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衛生福利部疾病管制署 104 年委託科技研究計畫

愛滋防治整合型研究計畫—
愛滋病防治中心
The HIV/AIDS Control and Study Center

年 度 研 究 報 告

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＊本研究報告僅供參考，不代表本署意見，如對媒體發布研究成果應事先徵求本署同意*

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中文摘要

研究目的：

愛滋病之防治、愛滋病患之照護與愛滋病學研究。

研究方法：

本年度延續 18 年來的工作，追蹤在台大醫院接受「高效抗反轉錄病毒療法」(Highly active antiretroviral therapies，HAART)治療的愛滋病患，包括其伺機性感染、臨床研究、及新病毒株的進行。本年計畫在人事穩定的基礎上，繼續活用本中心之軟、硬體，發揮本中心之特性，以臨床醫療服務為主軸，基礎研究及行為科學為輔，加強門診對病患之服務，改善併合療法及藥物副作用之研究。

依據聯合國愛滋病組織（UNAIDS）發表的最新報告顯示愛滋病毒(HIV) 感染人數逐年創新高，其中性行為與毒品是最主要的感染途徑，全球男女感染比率目前已趨近 1：1，因懷孕導致小孩垂直感染的案例日增，亦即可能有更多愛滋寶寶將由母親處感染到愛滋病毒。

在台灣，HIV/AIDS 的漫延稍晚，但其感染率仍然呈現逐年增加的趨勢，至 104 年 9 月底止，本國籍 HIV 感染通報人數共 30,432 人，已造成 4,909 人死亡。一直以來的愛滋病毒傳染途徑 90%係經由性行為傳染，毒品施用者因共用針具注射行為造成愛滋病毒感染長久以來為個位數，但自民國 92 年毒癮者感染人數首次突破十位數達 74 人，至 96 年 6 月已累積至 5,488 名，佔總感染人數 38.94%，顯示疫情逐漸飆升，面對此一新挑戰，幸賴行政院核定「愛滋病減害計畫」以因應毒癮愛滋個案的遽增，94 年 8 月由台北市、台北縣、桃園縣、台南縣進行「毒品病患愛滋減害試辦計畫」；並於 95 年 7 月起擴大辦理清潔針具計畫，全國 23 縣市共計設置清潔針具及愛滋衛教諮商輔導站 427 處；於 95 年 8 月擴大辦理替代療法計畫，全國 22 縣市設置替代療法醫療執行機構，使得毒品注射群體愛滋病盛行率顯著下降，但男同性戀族群間的愛滋病毒感染率仍然每年新增比例都超過 15%。再者，台灣與東南亞國家和中國大陸等高感染盛行率地區的交流頻繁，更加速 HIV 感染的擴散。

政府於 79 年 11 月 30 日經立法院三讀通過「後天免疫缺乏症候群防治條例」，同年 12 月 17 日由總統公布實施，中間歷經數次修訂施行細則以符合實際需求。90 年年底行

政院衛生福利部(前衛生署)為有效推動愛滋病防治計畫，減少感染人口，維護國人健康和確保青壯人口生產力，以防範愛滋病流行造成社會和經濟動盪，故凝聚各部會之力量共同推動，以統籌落實各項因應措施，特別設置了「愛滋病防治推動委員會」，將愛滋病防治提昇至中央跨部會之層級。為確保對愛滋病防治的重視，行政院承諾將愛滋病防治經費列為國家預算優先編列項目，加強跨部會協調與合作，盡一切努力，積極推動防治工作，全面對抗愛滋病漫延。台大醫院充分瞭解政府相關單位打擊愛滋病的決心，故結合本院與北區熱心同仁一起籌組「愛滋病防治中心」，以全體之力與政府共同對抗愛滋病帶來的挑戰。

台大醫院「愛滋病防治中心」於 86 年 6 月間成立以來，全體同仁積極參與防治與臨床工作，陣容愈來愈強化，不但責無旁貸地照護住院病患，亦主動且積極地派遣醫護人員前往各家醫療院所支援。本中心 18 年來在衛生福利部疾病管制署大力資助下已達到初期的成果，不論是臨床醫療服務或是研究工作皆成果豐碩，在人事訓練及佈局都已漸穩定，中心實驗室已稍具規模，將踏實地邁入繼往開來承先啟後的關鍵期。我們責無旁貸將繼續擔負起愛滋病防治與醫療的重要責任，所以向衛生福利部疾病管制署提出申請「愛滋病防治中心」第四期 3 年計畫，因為愛滋病毒的傳播涉及社會文化、性行為改變，實是一社會改造運動，而非單純醫療衛生問題，所以除了愛滋病醫療照護外，本中心將結合教育、文化、社會各體系共同合作推動防治計畫，尤其注重教育訓練，預定將以本中心及北區各個醫院之現有資源，開辦一系列衛教課程及研討會，因為普及防治教育和宣導，是打破 HIV 感染惡性循環的最佳方法。

我們體認到愛滋病不僅是致命疾病，更嚴重影響社會發展、減低生產力、讓外資怯步、降低人民生活水準，甚至削弱政府與社區力量。根據目前 HIV 感染增加率估算我國因愛滋感染之健保醫療累計支出將逐年增加，而薪資損失、社會福利等其他社會成本比健保支出更高數倍以上。若以實際 HIV 感染人數計算，其社會損失更為驚人。本中心的全體醫護同仁將提供愛滋病病患最適當的抗病毒藥物和伺機性感染藥物治療，每年更新用藥的準則，另外討論檢驗項目及間隔時間，訂定檢驗項目的原則，讓臨床醫師在照顧病患時有所遵循，並力行節約健保資源，研究更為經濟之治療方式來造福愛滋病患者，並強化照護系統，使病患、家人和其社區都能獲得適當的支持。

為提昇及結合全國愛滋病指定醫院醫療資源及感染者相關資料，進行全國性跨醫院之 HIV 臨床流行病學相關研究，協力從事包括了解國內感染者臨床特徵、伺機性感染治

療與預防、就醫意願、高危險行為、治療之抗藥性及副作用等相關臨床流行病學研究，以供後續治療與防治相關政策制定與修訂之參考。另「愛滋病防治中心」應扮演領導國內治療與防治相關之角色，應有相當之資源規劃教育訓練及建置並執行 PP line 等項目。

「愛滋病防治中心」104 年度計畫的實施重點如下列：

中程綱要計畫：愛滋病防治整合型研究計畫

分項策略：愛滋感染者臨床醫療照護利用與品質提升研究

研究重點：愛滋病防治之相關措施及治療照護與專業醫療人力培訓應用研究

一、研究內容包括：

主題一、愛滋病毒體液暴露者預防性用藥（Post Exposure Prophylaxis）：提供衛教諮詢、檢驗、預防性投藥與追蹤等 24 小時專線服務，並推展至特定易感族群如男男間性行為者或年輕族群等之可行方案產出與效益評估。

主題二、結合全國愛滋病指定醫院醫療資源及感染者相關資料，協力進行長期研究與監測國內感染者的臨床病徵，並提供每名感染者之抗藥性檢驗報告，另應依傳染病防治法規定，將感染者之檢體按月寄至疾病管制署。

主題三、愛滋藥物血清濃度監測，建立本土資料庫，監測其抗藥性情況等。

主題四、監測並分析感染者使用藥物之治療成效；另研究學名藥與原廠藥可能造成的影響。

主題五、辦理不同易感族群（如年輕族群、手機約炮及娛樂性用藥族群等）以雞尾酒療法治療、預防性投藥或結合精神科專業解決個案使用娛樂性藥物等預防感染或減害措施之影響因素分析、醫療利用性之研究，發展相關醫療照護模式以及有效之預防介入措施。

主題六、愛滋病毒感染者合併感染症（如結核病、肝炎、性傳染病等）相關流行病學與臨床治療研究與監測。

主題七、辦理醫事人員愛滋病治療照護及全面性防護措施等相關在職訓練課程至少 2 場、專題研討會至少 25 場。

二、研究目標：藉由調查及實驗室分析不同之易感族群之醫療利用特性，結合全國愛滋病指定醫院協力從事愛滋病防治相關之

1. 流行病學與臨床治療研究及監測。

2. 專業人員培訓及感染者預防計畫，以發展更佳之醫療照護及防治之介入模式。

3. 提供健全愛滋病醫療照護及防治策略之建議。

三、研究對象：感染愛滋病毒者及相關工作人員。

四、研究說明：

1.自 73 年通報第 1 例愛滋病毒感染者以來,截至 103 年 7 月累計本國籍通報個案數為 27,799 人，其中存活個案數為 23,376 人，約佔通報個案之 84.09%。

2.自抗愛滋病毒雞尾酒療法（HAART，Highly-Active Anti-Retroviral Therapy）治療問世以來，感染者生活品質提高，存活時間大大延長，但存活之感染者將日益增加，新感染者仍不斷產生的情形下，與感染 HIV 相關醫療問題，如相關合併感染症、治療副作用及抗藥性等醫療照護問題，需持續透過相關臨床研究與監測及醫事人員培訓與教育等，以提升治療成效與照護品質，並加強感染者衛教諮詢服務及行為介入措施，以避免其感染他人。

3.本計畫內容包含研究不同易感族群以不同雞尾酒療法藥物治療，以及保險套使用等預防感染措施因素，分析出具有意義之醫療照護模式以及有效之預防介入措施；評估愛滋病毒體液暴露後預防性用藥（post-exposure prophylaxis, PEP）之可行方案與效益等；藉由其研究及應用過程，培訓相關專業醫療、臨床、研究人才，提升愛滋相關實驗室及臨床研究質量。

「愛滋病防治中心」104 年度已完成 15 個子計畫，主辦3 場次大型在職教育訓練課程及研討會、2 場次 workshop、3 場次中小型研討會，發表 19 篇學術論文，參加 3 次國際會議發表 7 篇論文。

關鍵字：愛滋病防治中心、高效抗反轉錄病毒療法、伺機性感染預防與治療、愛滋病毒體液暴露後預防性用藥、醫事人員愛滋病治療照護及全面性防護措施

Abstract

Research Objective:

To study practices in AIDS control, AIDS patient care and treatment, and AID related studies.

Research Methodology:

This year is a continuing work implemented during the Phase I, the five-year Phase II, and three-year Phase III programs, and we shall track conditions of AIDS patients that underwent the Highly Active Antiretroviral Therapies (HAART) at the National Taiwan University (NTU) Hospital; at the same time, we shall also study the related opportunistic infection, clinical researches, and new virus strains. The program of this year, based on the established human resource foundation, shall see to the continuous utilization of the software and hardware of the Control Center to further develop the Control Center's features, and to focus the work on clinical treatment services, which is to be supplemented by fundamental studies and behavioral science. The program shall also see to the enhancement of outpatient treatment service, improvement of the integrated therapy, and study on the pharmaceutical side effects.

Since the establishment of the HIV/AIDS Control and Study Center of the NTU Hospital in June 1997, every member of the staff dedicatedly applied themselves to the AIDS control and clinical operations of the center, and the Center has continued to build a formidable human resource. Under the strong support of the Center for Disease Control of the Ministry of Health and Welfare (before was DOH) in the past eighteen years, the NTU AIDS Control Center managed to achieve the astounding accomplishments for its initial period work in both clinical treatment service and fundamental research. The human resource training and distribution of the Control Center has reached a certain level of stability, and the Control Center lab operations have achieved a certain scale. We expect to see sound progress towards the key periods of the course of the program. The Control Center adheres to the serious responsibility in the campaign for AIDS control and treatment since the proliferation of AIDS is closely related to the social culture and the change of sexual practices. Hence, it is a true social reform. The AIDS problem could not be solved by mere medical treatment and public health policies. Hence, in addition to providing AIDS treatment and nursing care, the Control

Center shall also implement the AIDS control campaign in cooperation with the educational, cultural, and social sectors. The campaign shall focus especially on health education. Moreover, a series of health education courses and seminars shall be held at the Control Center and the hospital facilities of the northern district for the popular dissemination of AIDS control education. This would be the most effective means by which we may break the vicious HIV communication cycle.

In response to the harm reduction policy of the government, the Control Center, under the leadership of Dr. Hung Chien-Ching, a team of young doctors and nurses from the Infectious Disease Department of Yunlin Branch Hospital take turns in conducting the following procedures every Friday at the Yunlin First Prison, the Yunlin Second Prison, and the Chiayi Prison: 1. Examine the newly diagnosed AIDS infected inmates or newly admitted inmates. 2. Understand the risk factors and current health conditions of the inmates, inform inmates of the relevant important health information, and answer health-related questions of infected inmates. Furthermore, conduct CD4/CD8 and AIDS virus count, Hepatitis A, B, and C viruses, liver function, and other basic biochemical tests. 3. Track the changes in the CD4/CD8 and/or HIV virus count and changes in the liver function; determine the time when sufferers should start taking HIV antiretroviral medicine. 4. Evaluate the methadone maintenance therapy aggressively implemented by the Yunlin Branch Hospital.

In an effort to upgrade and consolidate the resources and AIDS sufferer related information of the various AIDS designated hospitals in the country, nation-wide cross-hospital studies on HIV clinical epidemiology had been conducted, and assistance had been provided to researchers conducting studies on clinical epidemiology related matters, such as, clinical symptoms of AIDS sufferers in Taiwan, treatment and control of opportunistic infection, inclination to seek medical treatment, high risk behaviors, drug resistance and side effects of therapies. Results obtained shall serve as reference for the future definition and subsequent amendment of AIDS therapy and control related policies. Moreover, the HIV/AIDS Control and Study Center takes on the key role in leading AIDS treatment and control related efforts in the country; hence, it should assume the responsibility of planning the HIV-related education and training of medical personnel in the country, as well as establishing and operating the PP line. Recently, under the lead of Dr. Sheng Wang-Hui, a unified HIV body fluid exposure incident treatment procedure had been established through the concerted efforts of the doctors, nurses, and medical technologists of the NTU Hospital

HIV/AIDS Control and Study Center, with the help of the Branch for Communicable Disease Control of the Taipei City Hospitals (Kunming Branch) to aid sufferers exposed to the HIV body fluid. A 24-hour HIV screening and a hotline information and health education service had been established in an effort to reduce chances of HIV infection and to alleviate the fears and anxieties of persons seeking advices. Furthermore, a fast and single HIV test channel is provided to facilitate the diagnosis of any HIV infection within 24 hours. Once HIV infection is determined, free preventive medicine is provided to the patient within 24 to 36 hours, and notification is sent to the proper health authorities for the institution of effective epidemic control.

We have completed 15 sub-plans. There were 3 large-scale education and training in this year. There were 19 papers been published and participated in 3 international conferences.

Keywords: HIV, AIDS, The HIV / AIDS Control and Study Center, Highly active antiretroviral therapy, HAART, Post Exposure Prophylaxis line / PP line.

(一)前言

1997年12月李登輝總統公佈實施之新「後天免疫缺乏症候群防治條例」[(1)]，其中第四條明文規定:「中央衛生主管機關應設專責機構，辦理本條例有關事項及後天免疫缺乏症候群之防治與研究」。基於撙節人力、資源之原則，在專責機構正式成立之初，先於1997年6月間，由台大醫院與前台北市立性病防治所(現台北市立聯合醫院疾病管制院昆明院區)先行辦理「愛滋病防治中心」第一、二期5年計劃暨第三期3年計劃，進行相關防治與研究事宜。

根據衛生福利部疾病管制署截至2015年9月底最新統計資料顯示[(4)]，國內累計愛滋病毒感染人數已達31,425人（本國籍為30,432人），目前已發病人數本國籍是13,630例。歷年來MSM的感染人數幾乎是呈現逐年增加的趨勢，從1985年的1人，逐漸增加到2015年9月份的14,897人。1988-1991年MSM之性行為主要之傳染途徑；1992-1995年異性戀間性行為則躍居主要傳染途徑；1996-2003年MSM又回到第一位。2004-2006年毒癮者共用針具取而代之成為主要傳染途徑，但因2005年開始執行減害計畫，2008年毒癮愛滋感染者大幅下降，MSM又再度躍居第一名，且近兩年來MSM感染者增加率皆大於10%。在年齡層分布方面，感染愛滋的年齡層以25至49歲最多，佔69.80%，其次為15至24歲，佔23.17%，兩者共佔全體感染者的92.97％左右，顯見青壯年是感染愛滋病的最大族群，且〝危險性行為〞及〝毒品使用〞仍是最主要的傳染途徑。

本國人士得到愛滋病毒感染之人數近年來因注射靜脈毒品感染者在 2005 年突然劇增而快速成長，幸賴 2008 年因減害計劃推動而大幅減少。壞消息是若扣除毒癮之新感染者，2011 年新通報之男同志愛滋病毒感染者比起 2004 年數目增加 2 倍，異性間性行為而感染者亦增加有 1.5 倍之多，意即每年新發現毒癮以外之新個案數仍然持續增加中。因此除了繼續推動減害計劃外，對於安全性行為之加強推廣尚需努力。台灣過去因性行為而感染到的是 B 及 A/E 亞型，但從 2004 年起經由注射毒品而新感染者突然大增，靜脈毒癮者其亞型與大陸類似，以 CRF B/C 亞型最多；因毒癮患者之男女比例較為接近，且部分女毒癮患者有出賣靈肉之情形，因而可能影響性行為而來之病毒亞型分佈。因此台灣愛滋病毒亞型之監測是今後必須持續進行之長期流行病學工作。

由上述之感染趨勢，估計目前已登記但尚不需治療之感染者達到CD4<300/cmm應開始HAART治療時，約是3-4年後，屆時每年會有超過1,000人。在HAART的治療下，愛

滋病之伺機性感染與腫瘤發生機會微乎其微；但在現實世界裏，不是所有感染者均會聽從醫囑， 部分病患根本不來定期檢驗，往往愛滋病發後方來就醫，此一情形在毒癮感染者極為常見。此外，一般認為真正已感染之人數往往是登記有案數目的兩倍以上，因此病發時才查到HIV抗體陽性者不在少數。這個現象反映在今年台大醫院因愛滋病病發(即伺機性感染或腫瘤)而住院者，竟有一半是病發後才被查出有愛滋病毒感染；這些病患可能因延誤而死亡，即使存活， 也是療程坎坷，社會成本與醫療成本更是浩大。未能及早治療成為愛滋病防治之一大隱憂。

為了使醫療界各機構對HIV/AIDS病患之處置與研究專責化、全面化，「愛滋病防治中心」必須更積極推展防治與研究工作，並擔負起統籌全國性HIV/AIDS防治、醫療與研究的重責大任[(2)]。

(二)材料與方法

實施期間為自民國 104 年 1 月 1 日起至 12 月 31 日止。本期最重要防治工作必須接續前 17 年的未完成工作，並擴大本中心之功能，其實施重點如下列：

104 年度實施重點如下列：

主題一、愛滋病毒體液暴露者預防性用藥（Post Exposure Prophylaxis）：提供衛教諮詢、檢驗、預防性投藥與追蹤等 24 小時專線服務，並推展至特定易感族群如男男間性行為者或年輕族群等之可行方案產出與效益評估。

主題二、結合全國愛滋病指定醫院醫療資源及感染者相關資料，協力進行長期研究與監測國內感染者的臨床病徵，並提供每名感染者之抗藥性檢驗報告，另應依傳染病防治法規定，將感染者之檢體按月寄至疾病管制署。

主題三、愛滋藥物血清濃度監測，建立本土資料庫，監測其抗藥性情況等。

主題四、監測並分析感染者使用藥物之治療成效；另研究學名藥與原廠藥可能造成的影響。

主題五、辦理不同易感族群（如年輕族群、手機約炮及娛樂性用藥族群等）以雞尾酒療法治療、預防性投藥或結合精神科專業解決個案使用娛樂性藥物等預防感染或減害措施之影響因素分析、醫療利用性之研究，發展相關醫療照護模式以及有效之預防介入措施。

主題六、愛滋病毒感染者合併感染症（如結核病、肝炎、性傳染病等）相關流行病

學與臨床治療研究與監測。

主題七、辦理醫事人員愛滋病治療照護及全面性防護措施等相關在職訓練課程至少2場、專題研討會至少25場。

為配合以上重大主題之推展，故擬規劃執行以下 15 個子計畫，其工作內容分述如下：

一、〝醫事人員愛滋病治療照護及全面性防護措施等相關在職訓練〞之實施：

為配合疾病管制局關於 HIV 指定醫事機構的指定原則文中第三條規定：醫療人員、藥師及個管師需有愛滋病學相關教育學分 8~10 小時，故規劃及執行各科醫事人員針對愛滋感染者照護之相關在職訓練，分北區、南區舉辦「醫事人員愛滋病治療之相關在職訓練課程」，此教育訓練課程將有初階及進階等不同的課程內容，參加對象為對於照護愛滋病患有興趣之醫療人員，並與疾病管制局合作宣導及鼓勵其他科別的醫師來參加訓練，籌劃跨科整合的訓練課程，擬包含感染科、婦產科、兒科、家庭醫學科、精神科、內科、外科、牙科、藥師等醫護人員及社工人員等，同時申請臺灣醫學會、台灣感染症醫學會、台灣婦產科醫學會、台灣兒科醫學會、台灣愛滋病學會、內科醫學會、台灣家庭醫學會等相關醫學會持續教育學分認證，以提高學員之參加意願，全程參加者結業時並頒發授課證明。104 年度擬定期於北區及中區舉辦 HIV 感染者病例個案討論會，由各區之指定醫療院所輪流提出各院診療之有意義及特殊的病例，和與會者一起討論。

此主題由台大醫院內科部感染科洪健清醫師執行，詳細研究成果參見附件一。

二、從事〝危險性行為後愛滋病毒感染之非職業性暴露後預防性投藥效果之前瞻性研究〞：

後天免疫缺乏症候群（愛滋病，Acquired Immunodeficiency Syndrome, AIDS），自從 1981 年在美國發現以來，已成為全世界二十一世紀最重要的公共衛生問題，國內自 1984 年首例迄今，已逾二萬三千名以上被診斷和通報，由於感染人數持續增加，而且年齡層逐漸下降，早已經是衛生署傳染病防治工作的重要課題之一。愛滋病是由人類免疫缺乏病毒（Human Immunodeficiency Virus, HIV）透過血液或體液接觸而所傳染，全球各地主要之流行途徑大多是經由性行為，因此亦為性病之一，防治之法無他，即倡導安全性行為之重要性，以及教導高危險群定期檢驗追蹤；已被感染者若能及早發現，一方

面需要追蹤治療，另一方面藉由 100%之安全性行為，防堵已感染者將愛滋病毒進一步傳播。因此呼籲經常無保護措施性行為者、且性伴侶眾多者接受篩檢與專業心理諮詢，是非常重要的。根據衛生署的統計資料顯示，平均每 2~3 個小時發現 1 名新感染者，因此提供一個可信賴的 HIV 體液暴露者篩檢與專業心理諮詢的管道，應是杜絕愛滋病傳播最重要而且有效的方法，並可讓感染者有及早接受治療的機會。此外，目前已有多項國外觀察性研究顯示，體液暴露後預防性投藥可減低愛滋病毒感染的風險，因此，我們還可針對特定高危險族群(如男同志)在發生性行為發生體液暴露後，除了提供諮商以外，我們還可以提供相關性病的檢驗和治療，並且進一步評估是否有需要建議使用暴露後預防性投藥以降低愛滋病毒傳染的風險。

本計畫所要達成的目標：

1. 建立並整合非醫療或警消環境中發生之愛滋病毒體液暴露事件處理流程，可發揮並強　化現行防疫功能。
2. 提供危險性行為後愛滋病毒體液暴露問題解決的管道，透過諮詢過程加強對傳染病防治的認識，積極採取預防措施，降低感染率。
3. 評估危險性行為後愛滋病毒體液暴露後預防性投藥之效益, 愛滋病毒體液暴露諮詢記錄及處理追蹤結果可作為相關單位及學術機構研究發展之用，作為爾後政策擬定之參考。
4. 針對特定族群推廣愛滋病毒預防感染之教育。

本計畫所要完成之項目：

1. 參考國內外文獻及指引更新愛滋病毒體液暴露後預防流程及藥物。
2. 提供快速愛滋病毒檢驗管道及風險評估，則於 48-72 小時內提供自費預防藥物
3. 針對高風險行為之求診者提供有關愛滋病毒體液暴露之教育及諮詢。
4. 定期舉辦研討會提供愛滋病毒體液暴露處理相關資訊。

此主題將亞東醫院內科部感染科楊家瑞醫師執行，其詳細研究成果參見附件二。

三、進行〝台灣地區原生性抗愛滋病毒藥物抗藥性的調查研究〞：

根據疾病管制署的統計，截至 103 年 8 月底為止，在台灣已累計有 28,965 人遭到第一型人類免疫不全病毒(HIV-1)的感染。目前，在台灣遭到人類免疫不全病毒感染的本國

病患，皆可在愛滋病指定醫院接受由衛生福利部疾病管制署公務預算支應的三合一雞尾酒療法。但隨著感染者人數逐年增加，藥費的支出也持續成長，為了兼顧財政預算及感染者的醫療權益，疾管署自 2012 年 6 月 1 日起實施「抗人類免疫缺乏病毒藥品處方使用規範」方案，建議臨床醫師按照該規範，優先開立價廉同療效之處方。由於優先處方包含非核苷酸類反轉錄腖抑制劑(nNRTI)，該藥物產生抗藥性的基因阻礙(genetic barrier)較低，有可能導致抗藥性病毒株的產生，進而影響藥物治療的效果。所以本計畫首先預定調查臺灣地區尚未使用抗人類免疫缺乏病毒藥物的五百位 HIV 患者的原生性抗藥性病毒基因盛行率，希望了解在該規範實施後，對於病毒基因型抗藥性盛行率的改變，及其對於臨床治療效果的影響。第二，由於接受雞尾酒治療的病人仍有一定比例的病人會有治療失敗的情形，為了幫助病人得到較好的醫療效果，及避免醫療資源浪費，我們也將提供一百個免費的抗藥性病毒基因檢測給治療失敗的病人，希望其報告結果可以幫助臨床醫師選擇適當的藥物，可以在最短的時間內將病人的病毒量抑制到可接受的範圍。由於目前許多新型的藥物已不斷地推出，我們除了分析蛋白酶(protease)及反轉錄酶(reverse transcriptase, RT)的基因變異，也將納入嵌合酶(integrase)及共受體選擇性(cell tropism)分析，以求提供臨床醫師全方位的藥物選擇。最後，我們將分析一些臺灣常見但國外較少見的基因突變，並整合這些病人的臨床資料，看這些基因突變與治療效果的關聯性。希望可以整理歸納出，在台灣地區，特定病毒基因變異與抗藥性產生的相關性。

由台大醫學院醫技系張淑媛教授擔任此計畫負責人，詳細研究成果參見附件三。

四、實施〝台灣地區 HIV 感染者藥品動態學和基因學研究〞：

抗愛滋病毒藥物（ART）的問世使愛滋病毒的感染受到良好的控制，減少後天免疫不全症候群（AIDS）的發生與死亡率。ART 中，非核苷酸反轉錄酶抑制劑（NNRTI）efavirenz 與 nevirapine、蛋白酶抑制劑（PI）lopinavir/ ritonavir、CCR5 抑制劑等主要經由肝臟酵素 cytochrome P450(CYP 450)代謝；PI 類 atazanavir 與整合酶抑制劑 raltegravir 主要經由肝臟尿苷二磷酸葡萄糖醛酸基轉移酶（UGT1A1）進行葡萄糖醛酸反應，上述酵素的活性影響 ART 的代謝速率，而酵素活性與單一核苷酸基因多形性（SNP）、生理病理狀況、藥品交互作用息息相關，因此每個人代謝藥品的速率可能差異極大，使藥品動態學（PK）的特性相去甚遠。代謝快時血中濃度降低，可能影響治療效果，甚至促使愛滋病毒產生抗藥性；代謝慢時，藥物之血中濃度提高，可能因此而增加療效、甚至增

加劑量相關的毒性。不僅 ART 有這樣的特性，治療 AIDS 相關之伺機性感染的抗生素如 rifabutin 與 trimethoprim/sulfamethoxazole 亦然，使得監測藥物血中濃度極具臨床價值。再者，目前多項 ART 已有學名藥上市，提供病人有效但價廉的選擇；監測藥物血中濃度可確保療效與安全性，在符合藥物經濟的原則下提供多一層把關。文獻中多使用高效能液相層析儀（HPLC）檢測藥物血中濃度，但並非每個醫療院所都能進行此種檢驗方式，因此有必要成立一個『藥品濃度監測中心』，協助各地的醫療人員監測 ART 與其他抗生素的血中濃度，並檢測相關酵素或 P-glycoprotein 基因型。本研究目的是藉由前瞻性的觀察，追蹤血中濃度、基因多型性、療效與副作用等關係，以探索最適合國人的 ART 劑量、藥品交互作用時之劑量調整原則等課題，累積國內之本土經驗，與國外文獻、臨床經驗相比較，不僅可增進病人用藥安全、達到最大的經濟效益，也能以論文期刊的方式與世界各國分享我國的用藥經驗。本實驗室近年來已建立監測 efavirenz、nevirapine、atazanavir、rifabutin 與 trimethoprim/ sulfamethoxazole 血中濃度的 HPLC 方法，共檢測多家醫院、超過千位服藥的愛滋病毒感染者。延續研究的結果不僅可確認國內成立 PK lab 的可行性、監測血中濃度的必要性及適當範圍，ART 與其他 HIV 患者常用之抗生素血中濃度的結果可提供臨床醫師調整劑量的參考資料，甚至做為衛生主管機關建議國人使用劑量或優先使用學名藥之重要依據。

由台大醫院藥劑部及台大醫學院藥劑系林淑文藥師負責執行此研究，詳細研究成果參見附件四。

五、HIV 臨床流行病學相關研究，將有〝接受合併抗病毒藥物之愛滋病毒感染患者發生低病毒量之多中心研究〞：

最近有越來越多的研究討論在開始接受高效能抗病毒藥物後無法達到病毒量測不到且無法檢驗抗藥性的這一群病人，不管在臨床上或是實驗室裡所代表的意義是甚麼。無法達到測不到愛滋病毒量但持續低病毒量的人可能在未來治療的過程中會有較高比率面臨病毒突變而治療失敗，因而導致 CD4 淋巴球的增加受到抑制。這當中有相當多的因素會影響愛滋病毒無法達到測不到的範圍，例如:病人服藥順從性、病毒抗藥性程度、藥物血中濃度等。

至於低病毒量該如何處理，目前尚無定論，但不同準則有不同的建議，有些建議把非核苷類反轉錄酶抑制劑(NNRTI)換成蛋白酶抑制劑(PI)，有些建議必須先加強病人服藥

的順從性或是做抗藥性測試。於是，本研究目的旨在推估在服用高效能抗病毒藥物後愛滋病毒量無法達到測不到範圍而持續低病毒量的感染者，是否在未來治療的過程中會面臨較高治療失敗機率或是 CD4 淋巴球的增加會較差。針對這些病人，臨床醫師可會選擇以下三種處理方式：維持目前治療處方，並加強藥物遵囑性的提醒; 將現有使用含 NNRTI 處方換成 boosted protease inhibitors;或者把目前處方換成 raltegravir 400 mg bid 和 Lopinavir/ritonavir 2 顆 bid 或者是 raltegravir 400 mg bid 和 darunavir (800)/ritonavir (100 mg) qd）。我們將針對這些處理方式的病患觀進行察，並追蹤 24 週和 48 週後檢測病毒可以達到測不到(40 copies/mL)的比率。

此計畫由署立桃園醫院內科部感染科主治醫師鄭健禹醫師，詳細研究成果參見附件五。

六、HIV 臨床流行病學相關研究，將有〝愛滋病毒感染患者使用 atazanavir 之抗愛滋病毒組合療法後產生膽結石及腎結石副作用之盛行率及相關影響因子〞：

台灣自2005開始引進atazanavir，但對於atazanavir所造成的膽結石及腎結石盛行率或是相關影響因子並不明確。因此，在本研究中，我們希望了解台灣愛滋病毒感染者在使用atazanavir或atazanavir/ritonavir做為抗愛滋病毒療法時，其膽結石及腎結石副作用及相關併發症的盛行率和發生率；並嘗試找出相關影響因子(如body-mass index [BMI]、肝硬化、atazanavir暴露劑量、期間及血中濃度)。本研究將調察2014年間，所有於本院追蹤的愛滋病毒感染者，在腹部超音波可以發現膽結石及腎結石的比例，我們將分析出現膽結石及腎結石發生的相關因子，是否和接受atazanavir或atazanavir/ritonavir等藥物組合相關。這些服用atazanavir或atazanavir/ritonavir等藥物組合的病患，有不少人接受血中藥物濃度監測和藥物代謝基因型檢驗，我們也會評估血中atazanavir藥物濃度是否和出現膽結石及腎結石發生相關。目前本院照護的愛滋病毒感染者約為2500人，而當中約有700多位愛滋病毒感染者接受atazanavir或atazanavir/ritonavir處方。

此計畫由台大醫院內科部感染科洪健清醫師負責主持，詳細研究成果參見附件六。

七、擬進行〝抗人類免疫缺乏病毒藥品處方使用規範效果分析〞：

我國自 1988 年起，政府預算提供感染者免費藥物治療，1998 年起則由健保局依重大傷病給付，2006 年起愛滋感染者的治療及藥費，改由衛生福利部疾病管制署每年編列

公務預算支應。依據中央健保局統計，2000 年愛滋感染者醫療費用為四億五千多萬元，2010 年全年愛滋經費支出增加至 22 億，2012 年政府支出約 30 億元，其中藥費約 25.8 億元。衛生福利部疾病管制署訂定「抗人類免疫缺乏病毒藥品處方使用規範」，自 2012 年 6 月 1 日起實施，以同療效但價格相對較低的處方作為優先選擇，目的希望能有效控制藥費支出。成本效益分析的結果，部份決定於所鍵入的參數，如藥物費用、副作用發生率、抗藥性的比率、更動藥物的比率、治療失敗的發生率及勞動成本等眾多因素，台灣的醫療成本、藥物規範、副作用發生率、抗藥性的型態與其他地區均有差異，國外的成本效益分析未必適用於台灣，本研究欲延續過去一年的資料，持續提供實施「抗人類免疫缺乏病毒藥品處方使用規範」後首次服藥病患的臨床資料，作為未來分析的依據。

由亞東醫院內科部感染科蔡茂松醫師主持此研究，詳細研究成果參見附件七。

八、追蹤進行〝合併 HIV 與 HBV 感染者使用 tenofovir 治療之長期追蹤研究〞：

台灣是 HBV 高流行的地區，HIV 感染者之慢性 HBs Ag 帶原率約為 16%，與一般非 HIV 感染族群的 B 型肝炎流行病學相似。Lamivudine 是一種可以同時抑制 HIV 與 HBV 的複製的核苷酸反轉錄酶抑制劑，然而使用 lamivudine 治療 HIV 感染者之 HBV 每年約有 12%產生 YMDD 抗藥性，一旦 HIV 感染者出現 HBV lamivudine 之抗藥性，後續發生急性肝炎發作的風險明顯增加，並且有較多機會發生肝硬化及肝癌。Tenofovir 也是一種可同時抑制 HIV 與 HBV 的複製的核苷酸反轉錄酶抑制劑，早期使用在 HIV 合併 HBV 感染者當發生 lamivudine 抗藥性突變時，作為第二線的抗 HBV 藥物；自 2011 年 6 月開始，tenofovir 也納入為 HIV 感染者第一線的抗病毒藥物選擇之一。本研究旨在監測分析 HIV 合併 HBV 感染者在使用 tenofovir 治療後，其 HBV 相關之臨床與病毒學療效，探討有無 lamivudine 抗藥性突變與 tenofovir 療效之關係，與傳統使用 lamivudine 治療之病患對照組比較；以及追蹤長期使用 tenofovir 治療之下，是否會造成 tenofovir 抗藥性突變之發生。研究期間預計納入：(1) HIV 合併 HBV 感染並有 lamivudine 抗藥性突變者，加入 tenofovir 作為抗病毒藥物組合，(2) HIV 合併 HBV 感染者第一線使用 tenofovir 作為抗病毒藥物組合，兩組病人進行分析比較。研究期間將定期追蹤患者之肝功能、HBV DNA 之變化、定量 HBsAg 之變化、HBeAg 及 HBsAg 的陰轉率以及 HBV tenofovir 抗藥性之發生率。希望探討對於 HIV 及 HBV 合併感染者治療 HBV 藥物之最佳處方，以減少患者發生 HBV 併發症，改善患者預後，期望藉由本研究提供臨床醫師做治療上之參

考。

由台大醫院新竹分院感染科李官燁醫師主持此研究，詳細研究成果參見附件八。

九、探討〝愛滋病毒感染者接種七價結合型肺炎疫苗五年後血清抗體變化〞計畫：

接種七價結合型肺炎鏈球菌疫苗已經在非洲被證實可以降低愛滋病毒感染者發生復發性侵犯性鏈球菌感染。美國成人疫苗接種委員會已經在 2012 年正式將結合型疫苗接種建議為十九歲以上的愛滋病毒感染的成人可以接種的疫苗。不過，愛滋病毒感染的成人接受了結合型肺炎疫苗後的長期血清抗體的變化相關研究仍然很少。我等在 2008-2010 年之間針對四百多位愛滋病毒感染的成人，提供免費七價結合型肺炎疫苗接種，初步 24 與 48 週的追蹤結果已經發表於 Vaccine 2012 (May)。到了 2014 年，受試者接種疫苗逐漸屆滿五年。我們將針對 115 對接種一劑或兩劑的受試者，定量五年以來的血清抗體效價，以評估是否有追加接種的必要性，並且評估和抗體消失或持續存在的相關因素。

此主題由台大醫院內科部感染科洪健清醫師執行，詳細研究成果參見附件九。

十、執行〝愛滋病毒感染者的梅毒發生率及相關因子探討〞計畫：

在高效能抗病毒藥物（HAART）廣泛運用於愛滋病毒感染治療的時代，愛滋病毒感染者仍有從事危險性行為的可能，使自己暴露在其他性病感染的風險中。因此本研究預調查愛滋病毒感染者梅毒感染發生率與相關危險因子的關聯性。本研究預採病歷回溯的方式，收集愛滋病毒感染者的臨床資料，包含梅毒檢驗價數、梅毒治療、定期 CD4 和血漿病毒量、病程中出現 B 型和 C 型肝炎感染與否及其使用抗病毒藥物之組合、藥物副作用等，並從個案管理系統調查相關危險性行為資料，以及利用統計學計算梅毒感染發生率與分析危險因子。

此主題由台大雲林分院內科部感染科林綺英醫師執行，其詳細研究成果參見附件十。

十一、觀察分析〝愛滋病毒感染者之糖尿病發生率及預後〞：

從開始使用抗愛滋病毒藥物後，愛滋病毒感染者的存活率大大的增加，隨之而來的是，抗愛滋病毒藥物相關的代謝症候群漸漸的被觀察到。在過去西方國家的研究當中，

愛滋病毒感染者得到糖尿病的發生率遠比一般族群來得高，在台灣的研究亦如是。過去的研究顯示，在愛滋病毒感染者中，糖尿病相關的危險因子，包括年紀、性別、家族史、肥胖與種族。因此本研究的目的是要了解在台灣，在愛滋病毒感染者中得到糖尿病的發生率與預後因子。我們將著手進行 18 歲以上所有愛滋病毒感染者的病例資料分析，時間從 2003 年 1 月 1 日至 2013 年 12 月 31 日。若病患在進入研究之前或當下已被診斷糖尿病，則將從此研究中排除。每位愛滋病感染者都追蹤至少一年，最終觀察日期為 2014 年 12 月 31 日。 我們將使用標準紀錄格式來收集病患的基本資料包括年紀、性別、體重、糖尿病與高血壓家族史、抽菸、喝酒狀況、藥物史(抗愛滋病毒藥物、降血壓藥、降血脂藥物)、糖尿病慢性併發症、B 型肝炎、C 型肝炎以及梅毒。各種生化標記包含血脂肪(T-CHO, TG, LDL-C, HDL-C)、血糖、糖化血色素(HbA1c)。愛滋病毒量、CD4 淋巴球計數、血清梅毒標記(RPR)亦同時記錄。糖尿病發生率、危險因子、糖尿病的慢性併發症與血糖控制將會在此研究中進行分析。

此主題由花蓮慈濟醫院感染科蔡人植醫師執行，其詳細研究成果參見附件十一。

十二、深入探討〝HIV 感染者帶狀皰疹發病率和水痘—帶狀皰疹病毒血清陽性率〞：

帶狀疱疹(俗稱皮蛇, HZ)是 VZV(水痘-帶狀疱疹病毒)再度活化的表現，這種病毒在初次感染時會引發水痘。初次感染之後，此病毒會潛伏在背根神經節或腦感覺神經節中，直到再度活化引發帶狀疱，可以是一個使人衰弱的疾病。在有高效能抗病毒藥物(HAART)之前，相較於非愛滋病毒感染者，愛滋病毒感染病患帶狀疱疹的整體年齡調整後的發病率約有 12-17 倍之高。 HAART 減少了最常見的伺機性感染的發生率; 然而，一些研究發現，它不能完全消除發生帶狀疱疹的風險，原因是由於 HIV 感染改變了細胞免疫。最近來自美國和德國的流行病學調查估計，接受抗病毒藥物的艾滋病毒感染者感染帶狀疱疹的風險仍然比一般人群高出 4-11 倍。台灣最近沒有這樣的流行病學調查。目前現有的流行病學調查是過去 1994-2003 年時所做的，那時是早期 HAART 時代，艾滋病毒感染者在台灣地區帶狀疱疹的發病率為 5.05 每 100 人年。更新愛滋病毒感染者之帶狀疱疹發生率和血清陽性率的研究是必要的，以確定長期使用 HAART 是否可進一步降低愛滋病毒感染者的帶狀疱疹發病率，並且評估是否可發展出針對愛滋病毒感染者帶狀疱疹的預防策略，這是值得研究的。此外，以往的研究顯示，和免疫功能正常的宿主相較，愛滋病毒感染病患帶狀疱疹表現往往較俱獨特性且病情嚴重，通常較難以處理。

愛滋病毒感染病患往往有反覆帶狀皰疹發作，多個個皮節參與，及同時有神經系統的併發症，如帶狀皰疹後神經痛和帶狀皰疹眼症。有鑒於愛滋病毒感染病患罹患帶狀疱疹較容易有併發症，且易發生於於較年輕的族群，目前還不知道是否有更多積極的具體策略適用於現今逐漸老化的愛滋病毒感染病患，雖然他們有較高的 CD4 淋巴細胞計數。對一般大眾而言，帶狀疱疹主要預防的策略是通過疫苗接種，以提高患者的免疫力，所以檢測愛滋病毒感染病患是否已俱有 VZV-IgG 抗體，是找出有罹患帶狀疱疹風險的愛滋病毒感染病患必要步驟。 Zostavax（減毒活病毒疫苗，比水痘疫苗 Varivax 多 14 倍的 Oka 株病毒）可以安全而有效地使用在這樣的高危險族群。

此主題由亞東醫院感染科鄭琬鵬醫師執行，詳細研究成果參見附件十二。

十三、著手研究〝愛滋病毒感染患者接種 A 型肝炎疫苗後長期血清抗體效價研究〞：

愛滋病毒感染者是感染 A 型肝炎病毒的高風險群。愛滋病毒感染者如果感染急性 A 型肝炎病毒，病毒血症和糞便中排出病毒的時間都較一般非愛滋病毒感染者高，這對於周遭親密接觸的朋友或親人，都會造成感染的風險。如果愛滋病毒感染者同時有慢性 B 或者 C 肝炎病毒感染，再感染 A 型肝炎病毒後，發生急性肝臟功能代償失調的風險大大地增加。因此，愛滋病毒感染者是被建議接種 A 型肝炎疫苗的對象之一。在 2009-2010 期間，我們在台大醫院針對愛滋病染(N=365)和未感染愛滋病毒(N=217)的男同性戀提供 A 肝疫苗接種。接種後追蹤到 72 周的結果已經發表(Tsang YT, et al. Hepatology 2013)。追蹤到 48 周，我們發現愛滋病毒感染者儘管接種三劑的 A 肝疫苗，其血清抗體陽轉率依然低於非愛滋病毒感染者(75.7-77.8% vs 88.5%)。再者，接種兩劑和三劑疫苗，對於愛滋病毒感染者的抗體陽轉率並沒有達到統計學上有意義的差異(75.7 vs 77.8%)。接種疫苗時，有比較高 CD4 和血漿中檢測不到愛滋病毒的受試者會有較高的抗體陽轉率。這些愛滋病毒感染者，在完成接種試驗後，大多持續在本院的門診追蹤。本研究是針對已經完成接種後追蹤五年的受試者，再次檢測每一受試者在每一年抗體持續維持陽性反應的比例。我們計劃評估接種兩劑和三劑疫苗的愛滋病毒感染者，對於維持抗體陽性率，在追蹤五年後是否有差異。

此主題由台大醫院內科部感染科洪健清醫師執行，詳細研究成果參見附件十三。

十四、著手〝接受全血丙型干擾素釋放分析檢測的HIV感染病患之結核病的發生率〞：

因皮膚結核菌素測驗有執行上的困難,所以丙型干擾素檢測為取代皮膚結核菌素測驗以偵測潛伏結核感染一吸引人的選擇。儘管如此，目前關於使用丙型干擾素檢測預測 HIV 病毒感染病患是否會從潛伏結核感染進展為活動性結核病的研究仍稀少。故本研究欲追蹤 617 位曾檢測過丙型干擾素的 HIV 病毒感染病患，以了解檢測結果和未來發生活動性結核病的關係，及檢測丙型干擾素陽性的相關因子，及發生活動性結核病的危險因子。

此主題由台大醫院內科部感染科孫幸筠醫師執行，詳細研究成果參見附件十四。

十五、進行〝接受高效能抗反轉錄病毒治療之後天免疫不全病毒感染患者合併 C 型肝炎病毒感染中接受長效型干擾素合併雷巴威林後之長期病毒預後研究〞：

針對後天免疫不全病毒及 C 型肝炎病毒共同感染的患者的自然病史於文獻中已經相當清楚。相較於 C 型肝炎病毒單獨感染的患者，後天免疫不全病毒及 C 型肝炎病毒共同感染的患者於罹患急性 C 型肝炎時較易形成慢性感染。於形成慢性 C 型肝炎後，後天免疫不全病毒及 C 型肝炎病毒共同感染的患者會有較高的 C 型肝炎病毒量，較高風險的 C 型肝炎傳染，較快速的肝臟纖維化惡化，以及較高的疾病併發症及死亡率。除了引發肝臟病變之外,共同感染後天免疫不全病毒及 C 型肝炎病毒尚可引發身體多系統的病變 (包含神經病變, 心臟血管病變, 腎臟病變, 代謝病變, 骨骼病變, 及腸胃道病變)。隨著高效能抗反轉錄病毒治療的引進，後天免疫不全病毒及 C 型肝炎病毒共同感染的患者於肝臟相關的疾病併發症及死亡率有明顯的改善。其中病患若以干擾素(IFN)為主的治療成功清除 C 型肝炎病毒後更可以改善整體存活率，意指有效應用 C 型肝炎抗病毒治療可以改善免疫不全病毒及 C 型肝炎病毒共同感染的患者的預後。

以長效型干擾素(Peg-IFN)加上雷巴威林(RBV)為慢性 C 型肝炎之治療選擇。此外當後天免疫不全病毒及 C 型肝炎病毒共同感染的患者接受治療達成持續性病毒反應(SVR)之後平均追蹤 3.9 年仍有高達 99%的病毒清除率。[17] 然而相較於 C 型肝炎病毒單獨感染的患者,後天免疫不全病毒及 C 型肝炎病毒共同感染的患者接受長效型干擾素合併雷巴威林治療其持續性病毒反應率僅有 27-50%。[18-23] 其中病患為 C 型肝炎病毒基因型 1 型或是 4 型的持續性病毒反應率較 C 型肝炎病毒基因型 2 型或是 3 型為差。近年來由於直接抗病毒藥物(DAA)合併長效型干擾素合併雷巴威林或是不使用長效型干擾素之治療策略使得後天免疫不全病毒及 C 型肝炎病毒共同感染的患者接受治療的持續性病毒反應大於 60%。除了病毒量,肝臟纖維化, 病毒基因型, 以及胰島素阻抗性之外，人類宿主

介白質 28B (IL-28B) 基因多型性對於影響治療反應扮演重要的角色。[26] 相較於西方人大多具有不良的人類宿主介白質 28B 基因，亞洲人大多具有優良的人類宿主介白質 28B 基因，意指亞洲慢性 C 型肝炎患者若以干擾素為主的治療可以達成更佳的持續性病毒反應率。

雖然後天免疫不全病毒及 C 型肝炎病毒共同感染的患者接受抗 C 型肝炎病毒後若達成持續性病毒反應時會有長期且持續的病毒清除率，然而此類病患於治療後並無終生免疫的現象。由於後天免疫不全病毒及 C 型肝炎病毒共同感染的患者共用針頭及危險性行為導致 C 型肝炎病毒再感染的風險較一般患者為高，因而此項問題更為重要且需要長期前瞻性的追蹤研究。此外經由治療後的患者其病毒反應與長期肝臟纖維化的動態演進仍不清楚，因此我們針對後天免疫不全病毒及 C 型肝炎病毒共同感染的患者接受長效型干擾素合併雷巴威林抗 C 型肝炎病毒後之長期病毒預後從事研究。

此主題由台大醫院內科部腸胃科劉振驊醫師執行，詳細研究成果參見附件十五。

(三)結果

一、〝醫事人員愛滋病治療照護及全面性防護措施等相關在職訓練〞之實施：

愛滋病毒感染相關的知識日新月異，新藥物與治療的研發蓬勃發展，面對這些醫療新知的獲取，對照護愛滋病患的醫事人員而言格外重要且迫切，故愛滋病防治中心扮演傳播及教育國內治療與防治相關知識的角色，積極且定期規劃及執行各科醫事人員「愛滋病治療照護及全面性防護措施等相關在職訓練」課程。

(1)、104 年 3 月 14 日於台北舉辦「2015 醫療人員 Post-CROI 研討會」邀請參與 2015 CROI 會議的愛滋病毒感染治療學者專家，在會後整理會議資料，有系統地介紹給國內的醫師和個管師。衛生署指定醫療院所照護愛滋病毒感染者之專責醫師及個管師共 35 位參與。

(2)、104 年 3 月 28 日舉辦「愛滋病毒跨院教育與研究」共集結國內臨床醫療及研究學者專家共 35 位一起討論有關「臨床照護教育及研究平台」，有兩項討論及 17 點回饋。

(3)、104 年 4 月 29 日舉辦「台灣急性愛滋病毒感染研究討論會」國內臨床醫療及研究學者專家共 17 位出席，有 6 項討論及 10 點回饋。

(4)、為配合衛生福利部疾病管制署對於指定醫療院所照護 HIV/AIDS 醫護人員之在職訓練要求，104 年 6 月 7 日(日)於國立成功大學醫學院第三講堂舉辦「藥師愛滋病治療專業能力教育訓練課程」，參加對象：任職後天免疫缺乏症候群指定醫療院所的藥師，參與減害計畫指定醫院或藥房之藥師，其他對於愛滋病治療有興趣的藥師。統計參會者共有 112 位藥師。

(5)、7月11、12日特別開立「2015全國愛滋病毒感染個案管理師教育訓練研習班」，共有國內指定醫師院所現職之臨床愛滋病毒感染個案管理師57位完成課程。

(6)、為提昇國內感染症專科醫師愛滋病治療專業能力及參與力，繼續與台灣感染症醫學會、台灣愛滋病學會，針對有興趣從事愛滋病治療的年輕感染症專科醫師開立「感染症專科醫師愛滋病治療專業能力教育訓練研習班」。參加對象為感染症專科醫師訓練醫院之第 4、5 年住院醫師，將於 104 年 9 月 12~13 日共 1.5 天，洽借公務人力發展中心福華國際文教會館舉辦，計有 52 位感染科住院醫師參加。

(7)、104 年 9 月 4~5 日於台南與成大醫院共同舉辦「104 年度台灣多中心愛滋病學術研討會 Taiwan HIV/AIDS Research Meeting」會中並邀請研究梅毒之比利時專家 Dr.

Chris Kenyon 專題演講，並與國內專家學者共同討論，計有 52 位專家學者參與。

(8)、9/7 日在台北由比利時研究梅毒專家 Dr. Chris Kenyon 及國內專家專題演講，「Symposium of Management of Sexually Transmitted Diseases」有 35 位醫療人員參與。

(9)、8/22 日 HIV Resistance(抗藥性)Workshop 基礎班有 44 位醫療人員參與，11/17 日的 HIV Resistance(抗藥性)Workshop 進階班預計有 40 位醫療人員參與。HIV 的治療需考慮許多因素，而抗藥性是治療中相當重要的一環。為了讓國內醫師更熟悉 HIV 及抗藥性的重要性，特別設計了一系列的課程，期望醫護人員於課程結束後，能在治療上更加得心應手，嘉惠更多病患，故舉辦此教育訓練課程。

(10)、10/31~11/1 日邀請英國具有HIV病患器官移植經驗的專家 Dr. Frank Post 專題演講，在台中舉辦「Post-IAS & EACS new strategy update & HIV transplantation issues update」有47 位醫療人員參與。

(11)、本中心延續以往每週一次的愛滋病研討會，固定于每週二早上在綜合病房研討室舉行，本年度聘請了各方面的專家來進行全方位的研討，其內容包括有臨床醫學、病毒學、免疫學、流行病學、護理學、精神科醫學、個案研究、研究成果發表及新抗病毒藥物之介紹等；參加成員亦日益踴躍，包括有各科各級醫師、護理人員、檢驗人員、助理人員、社工人員、各基礎學科教師，踴躍參與，以期大家能各憑專業集思廣益。本年度1~11月份已進行26場。(詳細成果內容如附件一)

二、從事〝危險性行為後愛滋病毒感染之非職業性暴露後預防性投藥效果之前瞻性研究〞：

由於本次前瞻性研究之 IRB 於 2015 年 05 月 05 日通過可正式收案，因此自 2015 年 05 月 05 日至 2015 年 10 月 31 日止，共有 50 人次至板橋亞東醫院接受暴露後預防性投藥，全部的人均在 72 小時之內投藥，完成 28 天投藥的比例為 100%。對於處方之藥物主要是以 TDF+3TC (或 TDF/FTC) +RAL 為主，其次則為 TDF+3TC (或 TDF/FTC) +LPV/r，大致上以 RAL-based regimen 副作用較小。追蹤至 2015 年 10 月底，並無血清陽轉成 HIV 感染者。尋求預防性投藥者大部份為男性，但男同性戀者約佔所有人的 72.0%，有 74.0%的人是透過網路尋求一夜情對象，另外則有 22.0%是找性工作者，92.0%的人學歷在大學以上，有 82.0%的人在過去三個月內至少有過兩個性伴侶，而這些高危

險族群中僅 48%曾經做過 HIV 篩檢，此外，有 64.0%在過去三個月內戴保險套的比例低於 50%。由於 nPEP 已推行數年，因此共有約 18%的個案過去曾經接受過預防性投藥。(詳細成果內容如附件二)

三、進行〝台灣地區原生性抗愛滋病毒藥物抗藥性的調查研究〞：

自今年 1 月 1 日起至今年 10 月 31 日止，自未接受過三合一雞尾酒療法的 HIV 感染者，我們一共收到 553 件血液檢體進行基因型抗藥性檢測，目前已完成 483 件檢體的 HIV-1 病毒基因型抗藥性分析。自三合一雞尾酒療法治療失敗的 HIV 感染者，我們一共收到 245 件血液檢體進行基因型抗藥性檢測，目前已完成 196 件檢體的 HIV-1 病毒基因型抗藥性分析。這些檢體來自全台各家醫院，其中 91.9%(444 件)來自北部醫院，7.7%(37 件)來自南部醫院，0.4%(2 件)來自東部醫院。在未接受抗反轉錄藥物治療的病人中，對任一類藥物具有抗藥性的病毒株其總體盛行率為 14.5%，對反轉錄酶抑制藥物(NRTIs)、非擬似核苷酸衍生物的反轉錄酶抑制藥物(NNRTIs) 及蛋白酶抑制劑 (PI)的抗藥性病毒株的比例分別為 4.6%、2.5%、及 15.2%。對兩種以上藥物具抗藥性的比例為 1.4%。在抗反轉錄藥物治療失敗病人檢體中，對任一類藥物具有抗藥性的病毒株其總體盛行率為 54.6%。對反轉錄酶抑制藥物(NRTIs)、非擬似核苷酸衍生物的反轉錄酶抑制藥物(NNRTIs)及蛋白酶抑制劑 (PI)的抗藥性病毒株的比例分別為 4.6%、37.2%、及 44.9%。對兩種以上藥物具抗藥性的比例為 31.1%。

由於在未接受抗反轉錄藥物治療的病人中，對任一類藥物具有抗藥性的病毒株其總體盛行率為 14.5%，相較於之前我們去年的調查，其抗藥性的比例有些微升高的趨勢(13.2% v.s. 14.5%,)，主要升高的抗藥性藥物種類是蛋白酶抑制劑 (PI) (2.5% v.s. 4.6%)及非擬似核苷酸衍生物的反轉錄酶抑制藥物(NNRTIs) (9.4% v.s. 15.2%)產生抗藥性。擬似核苷酸衍生物的反轉錄酶抑制藥物(NNRTIs)及多重抗藥性病毒株的盛行率分別是些許下降(3.0% v.s. 2.5%)及 (1.6% v.s. 1.4%)。在接受抗反轉錄藥物治療失敗的病人中，對任一類藥物具有抗藥性的病毒株其總體盛行率為 54.6%，相較於之前我們去年的調查，其抗藥性的比例有些微下降的趨勢(61.2% v.s. 54.6%,)。蛋白酶抑制劑 (PI) (7.1% v.s. 4.6%)、擬似核苷酸衍生物的反轉錄酶抑制藥物(NNRTIs) (46.5% v.s. 37.2%,)、非擬似核苷酸衍生物的反轉錄酶抑制藥物(NNRTIs) (45.9% v.s. 44.9%)及多重抗藥性病毒株的盛行率(34.1% v.s. 31.1%)都有下降的趨勢。(詳細成果內容如附件三)

四、實施〝台灣地區 HIV 感染者藥品動態學和基因學研究〞：

本研究發現 EFV、NVP、ATV 與 cotrimoxazole 的個體間濃度差異性大、且部分病人的藥物血中濃度在建議值之外，有需要進行常規的血中濃度監測；針對 ATV 濃度過低者，應考慮加上 ritonavir 併用以提高 ATV 血中濃度、確保療效，但需密集監測總膽紅素數值，以避免副作用。而 EFV 劑量若依血中濃度修改，可避免副作用並達到較佳的經濟學效益。至於針對部分病人的 NVP 劑量是否需考慮調降，則須累積更多經驗後才能提出具體建議。監測 ART 血中濃度的結果可提供臨床醫師做為調整劑量或換藥的重要參考資料，研究成果不僅可用以確認國內成立 PK lab 的可行性、監測血中濃度的必要性，甚至做為衛生主管機關建議國人使用 ART 劑量時的重要依據。

在本研究室將繼續現行模式，除了提供全國各醫療院所常規監測 ATV、EFV、NVP、rifabutin 與 cotrimoxazole 血中濃度的服務、嘗試開發以 HPLC 方法測定其他 ART，在進行臨床相關研究的同時，亦提供臨床醫師重要的藥品濃度訊息。(詳細成果內容如附件四)

五、HIV 臨床流行病學相關研究，〝接受合併抗病毒藥物之愛滋病毒感染患者發生低病毒量之多中心研究〞：

截至2015年10月為止，一共有208位病人被收錄此觀察性研究，其中142位(68.3%)為觀察組不作處方上的調整，66位(31.7%)為實驗組，把原有的處方置換成不同機轉之抗病毒藥物 。這兩組的背景資料差別主要是實驗組有較多的靜脈毒癮的病人(12.3 vs 28.1%)，而當低量病毒量被偵測到時其組別間的病毒量中位數分別為(86 vs 149 copies/mL)，且病毒量小於200 copies/mL的比率分別為(84.0 vs 58.7%)。在觀察組中有42位(29.6%)繼續使用2種 (NRTIs) 加上1種 nNRTI; 30位(21.1%)使用2 NRTIs加上1種 PI；62位(43.7%)使用2 NRTIs加上 boosted protease inhibitor，而8位(5.6%)病人使用2 NRTIs加上1種 integrase inhibitor。在實驗組中2位(3.0%)處方被替換成2 NRTIs加上1種 PI；58位(87.9%)被替換成2 NRTIs加上boosted protease inhibitor；3位(4.5%)被替換成6 NRTIs加上1種 integrase inhibitor，而3位(4.5%)使用boosted protease inhibitor加上1種 integrase inhibitor。在多變相的分析中發現靜脈毒癮者([AOR], 6.81; 95%CI, 2.53-19.87)和病毒量在200-999 copies/mL的病人(AOR, 4.92; 95%CI, 2.01-12.48)有較高的比率被替換處方。在48週的觀察中發現，實驗組比對照組有較高比率可以達到HIV病毒量小於40

copies/mL(86.8% vs 66.5%, P=0.011)，但在病毒量小於200 copies/mL的比率兩組間沒有差別(96.6% vs 92.2%, P=0.635)。(詳細成果內容如附件五)

六、從事〝愛滋病毒感染患者使用 atazanavir 之抗愛滋病毒組合療法後產生膽結石及腎結石副作用之盛行率及相關影響因子〞：

截至 2014 年 12 月為止，一共有 910 位愛滋病毒感染者接受腹部超音波檢查。910 位愛滋病毒感染者之基本及臨床資料。男性居多(96.9%)，平均年齡為 42 歲，BMI 為 22.9 kg/m^2，已使用抗愛滋病藥物者有 85.8%。其所使用與核苷酸反轉錄酶抑制劑併用之抗愛滋病毒藥物，分別為非核苷酸反轉錄酶抑制劑(49.3%)、atazanavir (34.4%)、lopinavir/ritonavir (20.4%)、及 atazanavir/ ritonavir (5.5%)。此 910 位愛滋病毒感染者之膽結石及腎結石盛行率。整體膽結石及腎結石為 18.8%；其中膽結石盛行率為 12.5%，腎結石盛行率為 8.2%。未使用及已使用抗愛滋病藥物病患，其膽結石及腎結石盛行率分別為 16.3%及 19.2%，並未達到統計上顯著差異。顯示單變量及多變量分析膽結石及腎結石相關因子結果。於多變量分析中，與發生膽結石及腎結石相關的因子分別如下：使用 atazanavir/ ritonavir 超過 2 年 (odds ratio [OR], 6.11; 95% confidence interval [CI], 1.05-35.52)、較低的 CD4 基礎值(OR, 1.00; 95% CI, 1.00-1.01)、減低腎絲球濾過率(OR, 1.03; 95% CI, 1.01-1.05)。顯示 180 位愛滋病毒感染者之 atazanavir 血中藥物濃度。其中 16 位有發生膽結石及腎結石，164 位則沒發生。有發生膽結石及腎結石之病患，相較沒發生之病患，其投與 atazanavir 12 小時後之血中濃度略高 (0.85 vs. 0.66 mg/L)，但未達統計上顯著差異。有檢測藥物代謝基因型之愛滋病毒感染者的單核苷酸多型性結果。於發生及未發生膽結石及腎結石的病人，其 UGT1A1*28 及 MDR1 2677T/A 的分布是相似的(UGT1A1*28：12.5% vs. 19.9%；MDR1 2677T/A：71.4% vs. 70.9%)。(詳細成果內容如附件六)

七、進行〝抗人類免疫缺乏病毒藥品處方使用規範效果分析〞：

自 2012 年 6 月(實施抗人類免疫缺乏病毒藥品處方使用規範)後，目前總共陸續收集 965 位 HIV 病毒感染者開始使用抗人類免疫缺乏病毒藥物，其中 97.3%為男性，年齡中位數[median (IQR)]為 30.5(25.9-36.9)歲，開始接受抗病毒藥物治療前，平均 CD4 淋巴球數[median (IQR)]為 288.8 (145.6-398.1) cells/ul，平均病毒量[median (IQR)]為 4.83

(4.43-5.27) log10 copies/mL。965 位使用抗病毒藥物病患中 35.2%(340)使用第一類處方，58.3%(563)使用第二類處方，3.0%(29)使用第三類處方，3.4%(33)需使用第四類處方。965 位使用抗病毒藥物病患中 65.0%(628)會至少更動一次處方，開始使用到更動處方時間間隔為[median (IQR)] 35(14-196)天，其中 20.2%(127)是因為臨床懷疑是藥物過敏相關的皮疹，13.9%(87)因為抗病毒藥物相關神經精神副作用而換藥，9.1%(57)產生無法忍受的腸胃道不適而更替藥物，10.8%(68)的病患因為血紅素下降/白血球低下而換藥，值得注意的是 10.4% (65)因為治療效果不如預期或是藥物的抗藥性(genotypic resistance)而改變處方，30.3%(190)則因為服藥方便性(藥物顆粒數和服藥次數)更替處方。使用卡貝茲(combivir, zidovudine+ lamivudine)超過 28 天的病患，平均血紅素相較於基礎值下降 1.5 g/dL (95% CI. 0.9~2.1)達統計學上的意義。扣除單純因為服藥方便性而更動藥物者(N=438)，起始處方的選擇與之後持續處方具有統計的相關性，使用第一類處方之後有 65.8%需要更動處方，使用非第一類處方作為起始處方之後有 32.7%需要更動處方，有統計學上顯著的差異 (Log-Rank p <0.001)；分為四類發現，使用第一類處方作為起始處方之後有 65.8%需要更動處方，使用第二類處方作為起始處方之後有 32.5%需要更動處方，使用非第三類處方作為起始處方之後有 65.4%需要更動處方，使用第四類處方作為起始處方之後有 36.4%需要更動處方，四組之間有統計學上顯著的差異(Log-Rank p <0.001)。(詳細成果內容如附件七)

八、追蹤進行〝合併 HIV 與 HBV 感染者使用 tenofovir 治療之追蹤研究〞：

截至 2015 年 10 月，針對具有 HBV lamivudine 抗藥性突變之 HIV/HBV 合併感染者共納入 33 位病患，平均追蹤 3.7 年；第一線使用 tenofovir 治療之 HIV/HBV 合併感染者則共納入 56 位病患，平均追蹤 2.4 年。兩組幾乎皆為男性 (100%及 98.2%)，帶有 lamivudine 抗藥性突變者平均年齡較大 (42±8 vs. 36±8 歲)，兩組病患 HBV 之基因型主要皆為 genotype B (84.8%及 83.9%)，且開始使用 tenofovir 治療前血中 HBV DNA 平均值 (6.1±2.1 vs. 6.0±2.2 copies/mL) 及 HBeAg 陽性率 (48.5% vs. 31.5%, *P* =0.17) 並無差異 (Table 1)。兩組各有 33 位及 52 位病患完成至少一年 (48 週) 的治療與追蹤，其中各有 75.8%及 86.5%的病患其血中 HBV DNA 已降至<128 copies/mL (測不到) (Figure 1&2)。在多變量分析中，惟有 HBeAg 陽性與經 48 週治療後血清仍可測得 HBV DNA 相關 (odds ratio, 11.36, 95% CI, 1.93-66.82, *P* =0.007) (Table 2)。兩組各有 1 位及 2 位病患

在治療 48 週後血中 HBV DNA 仍 >1000 copies/mL，而同時間點 HIV viral load <40 copies/mL，目前未檢測到 tenofovir 相關之抗藥性突變。持續追蹤到治療後第二至五年，兩組病患血中 HBV DNA 曾達到測不到的比率分別為第二年：86.7% vs. 95%、第三年：92.6% vs. 96.6%、第四年及第五年：100% vs. 100% (Figure 2)。所有病患中只有 1 位病患在使用 tenofovir 36 週時發生血清肌酸酐上升而需停藥，其餘病患沒有發生任何嚴重副作用需要停用藥物。追蹤期間沒有任何病患發生 HBV 相關的急性肝炎。(詳細成果內容如附件八)

九、探討〝愛滋病毒感染者接種七價結合型肺炎疫苗五年後血清抗體變化〞計畫：

截至 2015 年 10 月為止，一共有 221 位預期觀察期間距離接種蛋白結合型肺炎鏈球菌疫苗後，已經達到五年的追蹤時間。其中 109 接種一劑，112 接種二劑。但是在一劑組中，有 7 位病患因為死亡(一位)，或轉介其他醫院追蹤、失去行蹤等，並未全程完成五年的血清追蹤。在二劑組中，也有 9 人轉介其他醫院追蹤或者失去行蹤。(詳細成果內容如附件九)

十、執行〝愛滋病毒感染者的梅毒發生率及相關因子探討〞計畫：

自 2009 年到 2014 年止，共有 3,102 位愛滋病毒感染者在臺大醫院追蹤及治療愛滋病毒感染，並在研究期間有追蹤梅毒血清學檢查(Rapid Plasma Reagin titer, RPR titer)；其中，有 2,798 位（90.2%）感染者之 RPR 檢測次數為兩次或以上，將其納入本研究發生率之計算與分析。在這 2,798 位愛滋病毒感染者中，有 820 位（29.3%）於研究期間有過一次或一次以上之梅毒感染，與沒有梅毒感染之個案相比，有梅毒感染的個案較年輕（平均年齡 32.5 歲 vs. 36.7 歲）、大多是男男間性行為者(87.3% vs. 69.9%)、B 肝表面抗體較多人呈現陽性（56.2% vs. 48.6%），而且梅毒血清效價在收案開始就呈現四倍或四倍以上者也比沒有梅毒感染者多（39.3% vs. 13.3%）。而有梅毒感染個案與沒有梅毒感染相比，兩組在 B 型肝炎表面抗原（13.7% vs. 14.8%）與 C 型肝炎抗體（7.3% vs. 9.3%）的盛行率，在統計學上均沒有明顯差異。在 820 位有梅毒感染之個案中，第一次發生梅毒感染後，有 68 位（79.1%）個案發生 C 型肝炎感染。自 2009 年至 2014 年為止，總觀察人年為 11019.1，梅毒發生率為 10.95 每 100 人年(95% CI, 10.34-11.57)；其中，2009 年至 2010 年止，梅毒發生率為 7.82 每 100 人年(95% CI, 6.73-8.91)，但在 2011 年至 2012

年止，梅毒發生率增加至 11.54 每 100 人年(95% CI, 10.45-12.63)，2013 年至 2014 年止，梅毒發生率更是高達 14.70 每 100 人年(95% CI, 13.59-15.80)。自 2012 年 6 月至 2015 年 7 月，在台大醫院就診之愛滋病毒感染者，有 870 位開始使用 HAART。自 2012 年 7 月 1 日至 2015 年 10 月 31 日，我們排除 15 位個案未服用 HAART 滿 30 天，並排除 59 位個案開始服用 HAART 超過 30 天、卻未在研究期間追蹤 RPR 價數，將剩餘 796 位開始服用 HAART 超過 30 天、有固定追蹤 RPR 價數的個案納入進一步統計分析。分析結果指出，198 位（24.9%）個案曾有過梅毒感染，梅毒盛行率為 24.87%；自 2012 年 7 月 1 日至 2015 年 10 月 31 日止，總觀察人年為 1190.4，梅毒發生率為 16.63 每 100 人年(95% CI, 14.32-18.95)。與沒有梅毒感染之個案相比，開始接受 HAART 治療後，有梅毒感染的個案較年輕（平均年齡 30.7 歲 vs. 33.1 歲），大多是男男間性行為者(98.5% vs. 92.6%)。與沒有梅毒感染的個案相比，有梅毒感染者在接受 HAART 治療前的 CD4 顆數及病毒量較高，但未達統計上顯著意義。有梅毒感染的個案當中，開始接受 HAART 治療後，第一次梅毒感染的平均年齡為 31.8 歲；開始接受 HAART 治療後，直到第一次梅毒感染的平均天數為 385.5 天；開始接受 HAART 治療後，90 天內發生梅毒感染則有 17 位（8.6%）。關於發生梅毒的相關行為因素目前仍持續使用個管資料分析中。(詳細成果內容如附件十)

十一、觀察分析〝愛滋病毒感染者之糖尿病發生率及預後〞：

從2003年1月1日至2013年12月31日在本院新通報之個案為1,712人，排除失聯個案為406人，新通報個案同時有糖尿病時的個案為6人，最後符合收案之個案為1,288人，這1,288位個案在同時追蹤至2015年10月31日為止。在1,288位個案中，總追蹤時間為7,071人年，共發現24位新發生糖尿病之個案，糖尿病的發生率為3.39/1000人年，總追蹤平均時間為5.5年，個案平均年齡為32.9歲，其中有1,256 (97.5%)為男性，32 (2.5%)位為女性。242 (18.8%)位在新診斷時同時有伺機性感染，161 (12.6%)位為B型肝炎表面抗原反應為陽性，81 (6.3%)位的C型肝炎抗體呈現陽性。在基礎點的CD4平均值為287 (cell/μL)，基礎點的病毒量平均值為4.88 $\log_{10}$(copies/mL)，基礎點的飯前空腹血糖為88 mg/dl。我們將個案進入研究的時間分為三個時段，分別為2004-2006年(204位)、2007-2009年(337位)、2010-2014年(747位)，三個時段的發生率分別為1.4/1000人年 (1/717)、2.6/1000人年 (3/1167)、1.2/1000人年 (3/2620)。三個時段的追蹤平均年為3.5年，我們發現在

2010-2014年診斷的個案相較於2004-2006年診斷的個案平均年齡較為年輕 31 vs. 36歲；基礎點的CD4，2010-2014年診斷的個案相較於2004-2006年診斷的個案平均數值較高 311 vs.196 cells/μL，基礎點的病毒量，2010-2014年診斷的個案相較於2004-2006年診斷的個案平均數值較低 5.1 vs.4.8 $\log_{10}$ copies/mL。(詳細成果內容如附件十一)

十二、深入探討〝HIV感染者帶狀皰疹發病率和水痘—帶狀皰疹病毒血清陽性率〞：

自2012年06月01日至2014年11月30日為止，共573位從未接受過雞尾酒療法之愛滋病毒感染個案在此追蹤時間內開始服用抗病毒藥物，並在研究期間有定期追蹤回診，個案回報或經醫師診療是否有發生Herpes Zoster，將其納入本研究發生率之計算與分析，資料之最後追蹤時間為2015年01月31日。在這573位愛滋病毒感染患者中，主要為男性病患(560位)，愛滋感染之主要風險為同性間性行為(535位,93.4%)，其中有33位感染者在研究期間內發生Herpes Zoster，發生率為每100人年3.86 (總觀察人年為854.19) 。發生Herpes Zoster者主要為男性病患且為同性戀者(32位)，平均年齡為34歲。把有發生Herpes zoster之33位感染者與沒有發生Herpes zoster之540位感染者分為兩組作比較，發現在研究期間內有發生Herpes zoster之感染者在開始服用抗病毒藥物前有較低之CD4數目(CD4 : 157 VS 277 cells/ul，p<0.17)，較高之血液愛滋病毒量(PVL: 5.17 VS 4.86 log10 copies/ul, p<0.002)，以及在服用抗愛滋病毒藥物後有較高幅度之CD4數目之增加(CD4增加: 114 VS 90.29 cells/ul, p<0.027)。(詳細成果內容如附件十二)

十三、著手研究〝愛滋病毒感染患者接種 A 型肝炎疫苗後長期血清抗體效價研究〞：

1.收案的流程圖和最後追蹤五年後的整體案數如附件十三圖一所顯示。2.在基礎點時，共有 140 人接種二劑和 225 人接種三劑 HAV 疫苗。3.依照 intention-to-treat 和 per-protocol 收納分析的個案數如圖一所示。4.隨著時間 HAV 抗體效價逐年下降，兩群受試者個別 geometric mean concentration 在每一年的 boxplot 如圖二所示。下降趨勢並未達到統計學上有意義的變化。5. 我們發現，在接種疫苗的第一年有產生抗體高於 20 mIU/ml 的受試者，經過五年後，根據 intention-to-treat 的分析方式，二劑和三劑依然能保有抗體數值高於 20 mIU/ml 的比例分別是 76.4% (84/110) 和 78.9% (146/185) 。6. 多變項的分析結果如附件十三表二所顯示。我們發現接種三劑的受試者較接種兩劑的受試

者有 8.09 倍(95% confidence interval, 2.414-27.123)的機會在第五年追蹤時依然能維持抗體效價高於 20 mIU/ml。(詳細成果內容如附件十三)

十四、著手〝接受全血丙型干擾素釋放分析檢測的HIV感染病患之結核病的發生率〞：

自西元 2011 至 2015 年 10 月 30 日止，共 617 位曾檢測過丙型干擾素的 HIV 病毒感染病患中，排除九位在檢驗當下有活動性肺結核的病患(一位檢驗結果為陰性，八位檢驗結果為陽)，剩下 608 位檢測當下沒有活動性肺結核的病患納入本研究，81%為男同志，15.7%為異性戀，1.5%為靜脈毒癮使用者，1.8%為其他族群。有 534 位(87.8%)檢測結果為陰性，64 位(10.5%)檢測結果為陽性，10 位(1.6%)檢測結果為無法判定。檢測當下，534 位檢測結果為陰性的平均 CD4 值為 441.6 cells/μl，平均病毒量為 2.89 $\log_{10}$ copies/mL; 64 位檢測結果為陽性病患的平均 CD4 值為 578.4 cells/μl，平均病毒量為 2.35 $\log_{10}$ copies/mL，追蹤至 2015/10/30 止，檢測結果為陽性的病患中，僅一位未接受高效能抗病毒藥物治療。10 位檢測結果為無法判定的病患，其平均 CD4 值為 473.5 cells/μl，平均病毒量為 2.79 $\log_{10}$ copies/mL。分析檢測結果陽性的相關因子為年紀較大者(調整後每增加十歲的勝算比為 1.172, 95%的信賴區間為 1.022-1.344, P=0.023)，過去有結核病病史者(調整後的勝算比為 13.412, 95%的信賴區間為 6.106-29.460, P<0.001)，及 CD4 值較高者(調整後每增加 50CD4 值的勝算比為 1.062, 95%的信賴區間為 1.017-1.109, P=0.007)。追蹤至 2015/10/30 止，僅一位檢測結果陽性患者沒有接受高效能抗病毒藥物治療。另一位檢測結果陽性患者在追蹤 337 天後發生活動性結核病，在結核病診斷當下，病患已接受雞尾酒治療，當時的 CD4 為 78 cells/uL，HIV 病毒量為 98 copies/mL。故 64 位檢測結果為陽性患者的結核病發生率為每 100 人年 0.422 件。 534 位檢測結果為陰性，在追蹤 1990 人年後，沒有人發生結核病。24 位檢測結果陽性且曾罹患過結核病的患者，在追蹤 88 人年後，沒有人發生結核病。(詳細成果內容如附件十四)

十五、進行〝接受高效能抗反轉錄病毒治療之後天免疫不全病毒感染患者合併 C 型肝炎病毒感染中接受長效型干擾素合併雷巴威林後之長期病毒預後研究〞：

1.收案的流程如如附件十五圖一所顯示。最終納入治療分析的人數，急性和慢性 C 型肝炎病毒感染的人數分別是 24 和 92 人。2.收納接受治療和分析的病患的基礎點相關臨床資料，如附件十五表一所示。 3.依據原先設定的急性或者慢性 C 型肝炎病毒感染

的治療療程，如圖二所示。4.急性和慢性 C 型肝炎病毒感染的愛滋病毒感染患者開始接受長效型干擾素和雷巴威靈後在第四週、第八週和第十二週時，血液中病毒量檢測不到的比例，如如附件十五表二所顯示。急性 C 型肝炎病毒感染者，全數都在第四週達到病毒量檢測不到，但是慢性 C 型肝炎病毒感染者僅有 54%達到(P<0.001) 。治療到第十二週時，病毒量檢測不到的比例，在兩組分別是 100%和 96% (P=0.53)。而在完治後追蹤 24 週時病毒量檢測不到的比例，在兩組分別是 83%和 72% (P=0.3)5. 基因型別和第四週、第八週、第十二週，以及在完治後追蹤 24 週時病毒量檢測不到的比例，分別顯示在表三中。6. 相關接受長效型干擾素和雷巴威靈過程中的副作用發生比例，如表四所顯示。7. 相關影響在治療結束後續追蹤 24 週能持續達到病毒量檢測不到的因子，在多變項分析的結果，如如附件十五表五所顯示。我們發現急性感染者，因為收納人數較少，我們無法找出可以達到統計學上有意義差異的因子。但是針對慢性感染者我們發現年紀(odds ratio [OR] per-1-year increase: 0.91, 95% confidence interval [CI]: 0.81-0.99, p=0.03)、高病毒量(OR per 1-$\log_{10}$ increase: 0.35, 95% CI: 0.14-0.84, p=0.02)、IL28B 基因型(OR: 5.56, 95% CI: 2.39-8.33, p=0.006)、和能在第四週即可達成病毒量檢測不到 (OR: 8.33, 95% CI: 5.89-14.3, p=0.003) 都是能預測在治療結束後續追蹤 24 週能持續達到病毒量檢測不到的因子。8. 不同基因型感染治療前能夠具有預測療效的因子, 如如附件十五表六所顯示。針對感染基因型第一或者第六型的慢性感染, 我們在多變項的分析發現具有預測的因子包含年紀 (OR per 1-year increase: 0.88, 95% CI: 0.78-0.99, p=0.04)、高病毒量 (OR per 1-$\log_{10}$ increase: 0.18, 95% CI: 0.03-0.98, p=0.03)、IL28B 基因型(OR: 5.52, 95% CI: 1.55-12.2, p=0.02 和能在第四週即可達成病毒量檢測不到(OR: 9.62, 95% CI: 3.89-15.3, p=0.007)。(詳細成果內容如附件十五)

十六、其他成果：

(A)、參與國際研討會、研究計畫或藥物試驗方面：

2 月 23~26 日本中心張淑媛教授及洪健清醫師帶領數位同仁參加美國華盛士頓舉辦之 22th Conference on Retroviruses and Opportunistic Infections (CROI)會議進行學術交流討論及發表 1 篇論文。

4 月 25~28 日本中心數位同仁代表參加丹麥哥本哈根舉辦之 the 25th European Congress of Clinical Microbiology and Infectious Diseases，本研究團隊共發表 4 篇海報論

文。

10 月 21~24 日本中心數位同仁代表參加西班牙巴塞隆納舉辦之 the 15th European AIDS Conference (EACS)，本研究團隊發表 1 篇口頭論文及 2 篇海報論文。如下列:

1. High incidence of early syphilis in HIV-infected patients seeking HIV care at a university hospital in Taiwan, 2009-2014.
2. Prospective observational study of effectiveness and rate of discontinuation of lead-in dosing of efavirenz-containing regimens in HIV-infected antiretroviral-naive patients in Taiwan.
3. Multicenter Surveillance of Prevalence of the 23S rRNA A2058G Point Mutation and Molecular Subtypes in Treponema pallidum in Taiwan, 2009 to 2014.
4. Cholelithiasis and Nephrolithiasis Related to Atazanavir - containing Regimens in HIV - infected Patients: Prevalence, Incidence, and Associated Factors.
5. Effectiveness of maintenance combination antiretroviral therapy using half-dose efavirenz plus 2 nucleoside reverse-transcriptase inhibitors with the guidance of therapeutic drug monitoring. (Oral presentation in EACS)
6. Hepatotoxicity and skin rash of non-nucleoside reverse transcriptase inhibitor (nNRTI) plus nucleoside reverse-transcriptase inhibitors in HIV-infected Taiwanese. (Paper presentation in EACS)
7. Tenofovir-Associated Nephrotoxicity and Hypophosphatemia in HIV-infected patients: A Single-Centre Cohort Study. (Paper presentation in EACS)

(B)、優秀中英文論文之發表：

「愛滋病防治中心」成立至今已屆 18 年，本中心同仁 18 年來合計發表了百篇以上的論文，涵蓋病毒學、免疫學、臨床診斷與治療、伺機性感染與惡性腫瘤，社會心理學等各層面。又於成立十週年之際，為了緬懷莊哲彥教授過去的開創與領導，暨感謝衛生福利部疾病管制署及台大醫院歷年來各級長官的支持與指導，將本中心同仁 10 年來愛滋病相關之優秀論文集結成冊印製，以供各界參考。此論文集依論文性質分成六大類如下—Section I：HIV Virology and Epidemiology and Host Responses ，Section II：Viruses and Host Responses，Section III：Opportunistic Infections and Malignancies，Section VI：Coinfections，Section V： Antiretroviral Therapy and its Complications，Section IV：Psycho-Social Problems。合計有 120 篇，每篇內容均具原創性、代表性及本土性，而且

大多數是發表於世界著名期刊上，冀望能提供給社會各界及醫護人員繼續教育之參考，也為同仁們辛勤的工作成果做一個註腳。

104 年度計有 19 篇論文發表，每篇內容均具原創性、代表性及本土性，明細如附表所列，篇篇都是傑作，且多數發表於世界著名期刊上，特附上已發表之論文影印本如附錄。

(C)、整合台灣地區 HIV/AIDS 多中心研究合作平台：

本中心為整合國內 HIV/AIDS 相關之研究，特別於 104 年度召開多次的「台灣地區 HIV/AIDS 多中心研究合作平台討論會」，其場次如下：

1. 104年3月14日於台北舉辦「2015醫療人員Post-CROI研討會」衛生署指定醫療院所照護愛滋病毒感染者之專責醫師及個管師共35位參與。
2. 104年3月28日舉辦「愛滋病毒跨院教育與研究」共集結國內臨床醫療及研究學者專家共35位一起討論有關「臨床照護教育及研究平台」，有兩項討論及17點回饋。
3. 104年4月29日舉辦「台灣急性愛滋病毒感染研究討論會」國內臨床醫療及研究學者專家共17位出席，有6項討論及10點回饋。
4. 104年9月4~5日於台南與成大醫院共同舉辦「104年度台灣多中心愛滋病學術研討會 Taiwan HIV/AIDS Research Meeting」會中並邀請研究梅毒之比利時專家Dr. Chris Kenyon專題演講，並與國內專家學者共同討論，計有52位專家學者參與。

(D)、有關經費使用方面：

在全體同仁的瞭解及共體時艱下，大家互相配合協調，發揮分工合作的精神，將有限的經費完全充分運用，本期最後之經費結餘為0元。其明細如下：

期　間	補助款實收	人事費	業務費	管理費	結　餘
104年1月~12月	9,450,000 元	7,278,437 元	2,071,563 元	100,000 元	$0

(四)討論

一、〝醫事人員愛滋病治療照護及全面性防護措施等相關在職訓練〞之實施：

本計畫在 104 年已主辦 3 場次大型在職教育訓練課程及研討會、2 場次 workshop、3 場次中小型研討會，各場次皆有醫療人員約60~112 人次參與，對國內愛滋病防治之醫療教育貢獻良多。明年度擬繼續舉辦。每週一次的愛滋病防治中心研討會，本年度繼續聘請各界的專家來進行全方位的研討，內容包括臨床醫學、藥學、病毒學、免疫學、流行病學、護理學、精神科醫學、個案研究、研究成果發表及新抗病毒藥物之介紹等；以後將加強個案討論，以期大家能各憑專業集思廣益互相交流。明年度擬繼續定期舉辦。(詳細成果報告詳附件一)

二、從事〝危險性行為後愛滋病毒感染之非職業性暴露後預防性投藥效果之前瞻性研究〞：

從目前初步的結果可以看出，暴露後預防性投藥在高風險族群中，具有一定的成效，就如同國外的觀察性研究一般。到目前為止，無個案真正 HIV 陽轉。一部份的原因可能是藥物本身的預防成效，但另一方面則也有可能是因單次性行為其實感染率就文獻統計而言並不高，再加上來源個案的感染狀態絕大多數不明，因此沒有 HIV 感染。然而，我們已經觀察到，重複接受 nPEP 的比例高達 18%，顯示我們仍需仰賴針對這一些個案的衛教諮詢，改變他們錯誤的觀念， 方能在預防上達到最好的成效，更進一步可結合目前國際上推行的暴露前預防性投藥。此外，如何針對網路一夜情進行宣導，減少高風險性行為的比例，將是未來在防疫上重要的一環。(詳細成果內容如附件二)

三、進行〝台灣地區原生性抗愛滋病毒藥物抗藥性的調查研究〞：

今年的追蹤研究發現，在未接受抗反轉錄藥物治療的病人中，對任一類藥物具有抗藥性的病毒株其總體盛行率為 14.5%，相較於之前我們去年的調查，其抗藥性的比例有些許升高的趨勢(13.2 v.s. 14.5%)。進一步的研究發現，具原生性人類免疫不全病毒(HIV-1)抗藥性基因盛行率的上升主要是針對蛋白胺抑制劑(PI)及非擬似核苷酸衍生物的反轉錄酶抑制藥物(NNRTIs)這兩類藥物。以 NNRTI 藥物為基礎之治療方案為目前世界上最常採用第一線 HIV-1 治療方案，同時為世界衛生組織(WHO)所建議之第一線雞尾酒療法藥物，同時也是台灣治療愛滋病人的第一線藥物。根據我們之前發表的論文評估，

具傳播性之抗藥性病毒株在臨床現象所造成之影響，我們發現即使是對於一種抗反轉錄病毒藥物具有低程度抗性的傳播性抗藥性病毒株(基因型敏感性評分≦2.5；GSS≦2.5)，比起不具有傳播性抗藥病毒株的治療效果在使用雞尾酒治療(cART)前兩年會導至大於五倍的治療失敗風險。此現象於接受以非擬似核苷酸衍生物的反轉錄抑制藥物(NNRTI)為主之雞尾酒療法的病患中特別顯著。根據這些前人的研究及本計畫的研究結果，我們建議目前如果要接受 NNRTI 抗反轉錄病毒藥物治療前，應立即進行抗藥性檢測；或是建議使用蛋白胨抑制劑。因為在一大型研究第一線的雞尾酒療法針對具傳播力之抗藥性病毒株的影響之調查中，Wittkop 等人發現，以蛋白酶抑制藥物為基礎並帶有促進劑Ritonavir 的治療方案相較於以 NNRTI 為基礎的治療方式在易感染或有潛力感染低程度抗藥性病毒的病患中擁有較佳病毒治療結果；因此，他們建議在抗藥性病毒株流行但無例行性基因檢測的區域應該考慮使用帶有促進劑的蛋白酶抑制藥物之第一線雞尾酒療法。(詳細成果內容如附件三)

四、實施〝台灣地區 HIV 感染者藥品動態學和基因學研究〞：

本研究延續前年度之研究成果，預定於本年度繼續協助臨床醫師監測 ART 血中濃度，並大量收納使用 EFV、ATV、NVP、rifabutin 與 cotrimoxazole 的病人，著手進行藥品血中濃度與代謝酵素基因型、治療預後、副作用的分析。

ATV 臨床使用時，雖比其他蛋白酶抑制劑較少發生代謝方面的併發症，但因經由肝臟酵素 CYP 3A4 代謝，可能因藥品交互作用而影響藥效；另一肝臟酵素 UGT 1A1 負責 ATV 的排除，ATV 與膽紅素競爭的結果可能造成高膽紅素血症，5%左右的病人更會產生明顯的黃疸症狀。國外研究者發現 ATV 的血中濃度是影響高膽紅素血症的重要原因之一，因此建議血中濃度維持在 0.15 - 0.85 μg/mL 之間。另一方面，ATV 時需飯後立即服用、避免與制酸劑或其他胃藥併服。加上目前初步分析發現過高的 ATV 血中濃度與嚴重總膽紅素血症相關，與其他國外文獻的結論相符。因此監測 ATV 血中濃度與避免藥品交互作用實屬必要。

EFV 一般的給藥劑量通常是 600 mg 睡前服用，以避免中樞神經相關的副作用。我們監測 EFV 的血中濃度顯示絕大多數病人的數值均高於 HIV 治療指引建議的 1 μg/mL，且四分之三的人用藥時可兼顧療效達成與副作用的避免。

EFV 主要經由肝臟酵素 CYP 2B6 代謝，其基因多型性在歐美、泰國、日本、印度

的研究結果均顯示會顯著影響 EFV 的血中濃度，而本研究觀察國人的追蹤結果也印證了這一點。為避免不良反應的產生，目前在極少數療效佳、EFV 濃度高的病人中，已嘗試將劑量降低成每日 300 mg（半顆）、密切追蹤病毒量與 EFV 血中濃度，觀察是否具有藥物經濟學上的優勢。

以 HPLC 定量 NVP 血中濃度的方式建立後，與文獻的建議濃度相比，接近九成病人的 NVP 血中濃度高於治療指引建議的 3 μg/mL。其中個體差異頗大，而過高的血中濃度通常都發生在 maintenance phase。因目前收案人數少，濃度相關副作用（如皮疹、肝炎）、治療效果與 NVP 血中濃度的相關性仍有待評估。本院於去年引進 NVP 長效藥錠，仿單中提到少數病人可能在糞便中發現藥錠殘骸，應不影響療效。但實際使用後，多位病人提到此現象，因此本研究特別進行血中濃度與臨床療效的探索。9 個月內共追蹤 244 位服用 NVP XR 的病人，其中 49 位 (20.0%)發現糞便中有藥錠殘骸。發現者的平均 CD4 值較無發現者高(612 vs. 495 cells/mm^3, P=0.002)、病毒量相近 (1.55 vs. 1.66 log10 copies/mL, P=0.42)。治療 12 週後，發現者的 12 小時 NVP 血中濃度雖有較低的傾向 (3.55 vs. 5.17 μg/mL, P=0.05)，但平均 CD4 值較無發現者高(626 vs. 547 cells/mm^3, P=0.06)、病毒量較低 (1.39 vs. 1.52 log10 copies/mL, P=0.07)，結果已被 PLoS ONE 雜誌接受。(詳細成果內容如附件四)

五、HIV 臨床流行病學相關研究，〝接受合併抗病毒藥物之愛滋病毒感染患者發生低病毒量之多中心研究〞：

低病毒量的抗藥性的檢測是目前有高度的技術門檻，而隨著病毒量檢測的儀器進步，有越來越多的病人被發現有這個現象。但是該如何處目前尚無定論，所以目前的準則都僅止於專家型的建議。我們進行這一個研究是想探討這個現象的盛行率高低，此外把現有的抗病毒藥物處方如非核苷類反轉錄酶抑制劑(non-nucleoside reverse transcriptase inhibitor；NNRTI)換成蛋不同機轉的蛋白酶抑制劑(protease inhibitors；PI)或是嵌入酶抑制劑(integrase inhibitor)，在我們的觀察中發現在長達一年的追蹤有做處方替換的病人其病毒量可以有較好的控制。但是這無法適用在所有的病人上，因為前提是必須改善某些病人的服藥順從性。此外也必須考慮的病毒的突變多樣化，然而如何克服後者的技術門檻須仰賴未來更多的研究支持和協助。(詳細成果內容如附件五)

六、從事〝愛滋病毒感染患者使用 atazanavir 之抗愛滋病毒組合療法後產生膽結石及腎結石副作用之盛行率及相關影響因子〞：

先前台灣針對健康族群的盛行率調查，膽結石為 4.6-10.7% ，腎結石為 7.4% 。此研究中，我們發現台灣愛滋病感染者，有較高的膽結石及腎結石盛行率。即使是未使用抗愛滋病藥物者，其膽結石及腎結石盛行率也相對較高，其原因可能與收納病人有一半以上為 B 肝及 C 肝感染有關 ；而是否與愛滋病本身疾病相關，則需要進一步的研究。膽結石及腎結石的發生有許多相關因子，包含年齡、性別及其他代謝相關因子。愛滋病感染者在過去文獻中，曾經報導與發生膽結石及腎結石相關的因子包含 indinavir 及 atazanavir。此研究中，我們也發現使用 atazanavir/ ritonavir 超過 2 年為膽結石及腎結石之相關因子，但卻與血中藥物濃度和藥物代謝單核苷酸多型性並無相關性。我們另外發現較低的 CD4 基礎值也與膽結石及腎結石相關，可能解釋原因為，過去文獻顯示較低的 CD4 與年齡相關併發症(如糖尿病、腎衰竭)有關。(詳細成果內容如附件六)

七、進行〝抗人類免疫缺乏病毒藥品處方使用規範效果分析〞：

研究結果顯示，考慮治療效果、藥物副作用發生率與嚴重程度、抗藥性的型態，此一規範對於病人的用藥安全效果、臨床治療效果、符合病患服藥的方便性及順從性具有爭議。病患對於藥物組合的臨床耐受性有所不同，參考 WHO 於 2013 年的治療建議及 2015 訂定的治療目標，Combivir 仍做為國內的治療優先選擇必須配合臨床工作人員注意相關的副作用，而一日兩次的服藥限制對於病患的服藥耐受性是一大考驗，長期的副作用則有待日後更長時間追蹤及更多的病患納入分析。(詳細成果內容如附件七)

八、追蹤進行〝合併 HIV 與 HBV 感染者使用 tenofovir 治療之追蹤研究〞：

研究結果顯示 tenofovir 對於帶有 lamivudine 抗藥性突變，及無 lamivudine 抗藥、第一線使用抗病毒藥物治療之 HIV/HBV 合併感染者，其治療效果一樣好，經 48 週治療後各有 75.8%及 86.5%的病患其血中 HBV DNA 可降至測不到的範圍 (<128 copies/mL)，而持續追蹤到治療後第二至五年，兩組病患血中 HBV DNA 曾達到測不到的比率分別為第二年：86.7% vs. 95%、第三年：92.6% vs. 96.6%、第四及第五年：100% vs. 100%。多變量分析中惟有 HBeAg 陽性與經 48 週治療後血清仍可測得 HBV DNA 相關，因此對於 HBeAg 陽性的 HIV/HBV 合併感染者，應長期追蹤 HBV 之治療效果。在藥物的安全性

方面，追蹤期間只有 1 位過去有糖尿病史的病人在使用 tenofovir 36 週時發生血清肌酸酐上升而需停藥，因此對於本身已有可能影響腎功能之慢性病患者 (如糖尿病、高血壓等)，或併用其他可能有腎毒性的藥物時，應注意定期抽血追蹤腎功能。(詳細成果內容如附件八)

九、探討〝愛滋病毒感染者接種七價結合型肺炎疫苗五年後血清抗體變化〞計畫：

蛋白結合型肺炎鏈球菌疫苗已經正式被建議使用於免疫功能缺損的病患，含愛滋病毒感染者。但是，免疫系統缺損的病患，應該接種的適當劑數、是否要追加接種等等，目前仍不清楚。國際間的研究大多著重於短期的抗體血清研究。我們的研究是少數追蹤較大族群的愛滋病毒感染者，並且大多數開始接種時或者在追蹤當中接受抗病毒藥物治療，維持較低的病毒量和較好的免疫功能，同時，也是極為少數持續追蹤五年的血清抗體反應的長期追蹤研究。我們定義的產生明顯的血清抗體反應，和過去我們一系列的研究一樣，同時追蹤的肺炎鏈球菌夾膜型的血清型，也是相同，因此我們未來有機會同時比較接種多醣體疫苗和蛋白結合型疫苗五年追蹤的抗體變化。這些資料將可提供國內或國際間對於愛滋病毒感染者的肺炎疫苗接種建議做為參考。此研究中，我們發現接種二劑的蛋白結合肺炎鏈球菌疫苗，較於接受一劑的受試者，在多變項分析中，在五年依然有較高的機會(79%)維持明顯的血清抗體反應。因此，純粹就血清抗體的量做為研究觀察重點，我們的研究支持愛滋病毒感染者接種兩劑蛋白結合肺炎鏈球菌疫苗。(詳細成果內容如附件九)

十、執行〝愛滋病毒感染者的梅毒發生率及相關因子探討〞計畫：

我們發現在 2009 年到 2015 年間在台大醫院追蹤的愛滋病毒感染者梅毒發生率持續增加。雖然大多數人都有接受抗愛滋病毒治療愛滋病毒，愛滋病毒感染獲得適當控制，梅毒的風險依然沒有降低。我們的研究顯示至少 25%的感染者，尤其是男同志，持續有高風險的性行為導致感染致梅毒。在感染梅毒時患者生殖器或者口腔肛門的潰瘍部位愛滋病毒量升高， 容易造成傳染他們的性伴侶，同時他們也容易被傳染肝炎病毒或者抗藥性的愛滋病毒，造成慢性肝炎或者增加後續治療的困難度。(詳細成果內容如附件十)

十一、觀察分析〝愛滋病毒感染者之糖尿病發生率及預後〞：

在我們的研究裡發現愛滋病毒感染者的糖尿病累計發生率為3.39/1000人年，此發生率與一個研究結果相似為瑞士(4.4/1000人年)，相較於其他研究較低分別為泰國的研究(5.0/1000人年)、D:A:D 研究團隊(5.7/1000人年)、美國的研究(47/1000人年)、美國針對女性的大型研究(25/1000人年)以及台灣羅一鈞的案例對照研究(13.1/1000人年)。本研究與其他研究結果的差異可能是我們的研究族群平均年齡較輕(32.9 vs 39-51歲)、男性佔多數(97.5% vs 56-73%)。本研究與兩個研究結果差異不大的可能原因是年齡較相近。本研究尚未分析個案服用抗病毒藥物的種類、時間，所以我們暫時無法知道藥物對於糖尿病可能的影響，在未來我們會再加入抗病毒藥物的種類及時間以增加資料的完整性。在國外的研究裡有發現長期使用抗病毒藥物也會增加代謝疾病的風險,因此我們將繼續觀察三年，以期能較精確推估發生率和發現其他相關因子。本研究結果共追蹤了7,071人年，共發現24位新發生糖尿病之個案，糖尿病的發生率為3.39/1000人年，研究期間分為三個時段，分別為2004-2006年、2007-2009年、2010-2014年，三個時段的發生率分別為1.4/1000人年、2.6/1000人年、1.2/1000人年。(詳細成果內容如附件十一)

十二、深入探討〝HIV 感染者帶狀皰疹發病率和水痘—帶狀皰疹病毒血清陽性率〞：

(1) 在有雞尾酒療法之時代，愛滋感染者發生帶狀疱疹的發生率比起過往已經有降低之情形，但仍比一般非感染者之發生率為高。

(2) 在開始服用雞尾酒療法之愛滋感染者中，若感染者在開始服用抗病毒藥物前有較低之 CD4 數目，較高之血液愛滋病毒量，以及在服用抗愛滋病毒藥物後有較高幅度之 CD4 數目之增加者，都有較高之機會發生帶狀疱疹。可考慮針對有以上特性之感染者，加強教育以及提醒，讓感染者更瞭解要甚麼情形要及早注意回診，接受適當之治療，避免發生併發症。(詳細成果內容如附件十二)

十三、著手研究〝愛滋病毒感染患者接種 A 型肝炎疫苗後長期血清抗體效價研究〞：

針對接受抗愛滋病毒藥物治療而且在基礎點呈現 A 型肝炎抗體陰性的愛滋病毒感染者施打三劑的 A 型肝炎疫苗，會較給予施打二劑疫苗，在完成五年的追蹤時，有八倍以上的機會，依然維持有高於 20 mIU/ml 的保護感染 HAV 的抗體效價。(詳細成果內容如附件十三)

十四、著手〝接受全血丙型干擾素釋放分析檢測的 HIV 感染病患之結核病的發生率〞：

在本研究中，接受丙型干擾素檢測的 HIV 病毒感染病患中，陽性結果的盛行率約為 10.5%。檢測陽性的患者中，在追蹤過程中僅一位發生活動性結核病，故陽姓預測率為 1.6% (1/64)。而 64 位檢測結果為陽性患者的結核病發生率為每 100 人年 0.422 件。過去文獻中，丙型干擾素檢測陽性的 HIV 病毒感染病患中，其結核病發生率為每 100 人年 0 到 4.621 件 [9-13]。相較於其他研究，本研究的結核病發生率(每 100 人年 0.422 件)算低，會有這些差異主要是因執行研究所在地結核病的發生率不同，納入研究的病患數不一致(從 50 到 909 人不等)，追蹤的時間不等(1.28 年至 2.97 年不等)，各研究中診斷偵測結核病的方法不一，再者，有些病患接受潛伏性結核病的治療，有些接受接受高效能抗病毒藥物治療(0-100%)，病患的 CD4 數(305-447 cells/uL)和 HIV 病毒數(1.85-4.8 $\log_{10}$ copies/mL)不同所致。為減少丙型干擾素檢測陽性的 HIV 病毒感染病患進展到活動性結核病，及早開始高效能抗病毒藥物治療及接受潛伏性結核病的治療為為兩項最主要的方法。但有不少病患在 HIV 感染末期才診斷出 HIV 感染。需更加強 HIV 的早期診斷及讓感染病患能留在醫療體系追蹤，已接受高效能抗病毒藥物治療，成功控制 HIV 病毒。(詳細成果內容如附件十四)

十五、進行〝接受高效能抗反轉錄病毒治療之後天免疫不全病毒感染患者合併 C 型肝炎病毒感染中接受長效型干擾素合併雷巴威林後之長期病毒預後研究〞：

在這個觀察研究中，我們發現台灣的愛滋病毒感染者併有慢性或者急性的 C 型肝炎病毒感染時，給予長效型干擾素和雷巴威靈，分別有 72%和 83%的病患能夠在治療結束後續追蹤 24 週能持續達到 C 型肝炎病毒量檢測不到。這個治療成果相較西方國家的雙重病毒感染者也接受相同的治療處方來得好。因為藥物高價位的因素，目前國內能夠接受新的 directly-acting antivirals 的病患人數勢必很少。絕大多數依然需要接受傳統的治療方式 因此本研究可以提供國內臨床醫師和疾管署相關單位參考。其次，因為急性 C 型肝炎病毒感染有較高的機會可以在接受傳統藥物治療即可治癒，臨床醫療人員在遇見病患出現肝功能異常，尤其又併有像梅毒的性病發生時應儘快提供 C 型肝炎抗體檢測和追蹤，以期能早點診斷急性 C 型肝炎病毒感染和轉介接受適當的治療，以降低繼續傳染他人的風險。(詳細成果內容如附件十五)

(五)結論與建議

一、本計畫在104 年已主辦3 場次大型在職教育訓練課程及研討會、2 場次 workshop、3 場次中小型研討會，各場次皆有醫療人員約60~112 人次參與，對國內愛滋病防治之醫療教育貢獻良多。為提昇國內 HIV/AIDS 臨床及學術研究，104 年舉辦 4 場「台灣地區 HIV/AIDS 多中心研究合作平台討論會」。會中互相討論做成許多共識與結論。明年度擬繼續舉辦。

二、就初步的結果而言，暴露後預防性投藥有其效果，但絕非單一之預防方式，而應整合將個案連結進入衛教諮詢的系統，方可在 HIV 防疫上收到好的成效；針對難以抑制慾望而反覆發生高風險行為者，可考慮建議轉為暴露前預防性投藥。此外，防疫政策上仍須針對網路尋求一夜情的族群進行預防政策及宣導的擬定。

三、進行〝台灣地區 HIV 感染者服藥前的愛滋病毒藥物抗藥性前瞻性研究〞，我們的結論支持，如果病人要服用以 NNRTI 為基礎的雞尾酒療法，應該在用藥前接受抗藥性基因檢測，以確保藥物治療的效果。

四、本研究雖發現住院的成人使用 SMX-TMP 的劑量低於仿單建議劑量，但大多數病人可達文獻建議之 TMP 治療濃度，因此推測成人可能不需依照仿單建議的使用劑量即可達到理想濃度。肝毒性和 SMX 濃度有顯著關係；電解質不平衡和藥物濃度與使用劑量之間也有顯著關係。病人血中濃度個體間變異性大，因此，監測藥物血中濃度將有助於避免副作用。由於兩藥之血中最高濃度及最低濃度有良好的線性關係，可利用臨床上較易取得之血中最低濃度去預測最高濃度。Rifabutin 只有一位病人檢測，血中濃度在正常範圍內。以上研究結果不僅可確認國內成立 PK lab 的可行性、監測血中濃度的必要性，ART 血中濃度的結果可提供臨床醫師調整劑量的參考資料，甚至做為衛生主管機關建議國人使用 ART 劑量時的重要依據。本研究室將繼續現行模式，提供全國各醫療院所常規監測 ATV、EFV 與 NVP 血中濃度的服務；並嘗試開發檢測其他 ART 的 HPLC 方法，追蹤病人的藥物血中濃度與肝臟酵素及 P-gp 的基因型、評估臨床療效與副作用，以期達到最佳療效並確保用藥安全。

五、我們的研究支持低量病毒量者接受處方替換成不同機轉的藥物可以改善病毒被控制的狀況。

六、我們的研究顯示台灣愛滋病感染者，有較高的膽結石及腎結石盛行率。其相關因

子為使用 atazanavir/ ritonavir 超過 2 年、較低的 CD4 基礎值、及減低腎絲球濾過率。但 atazanavir 血中藥物濃度和藥物代謝單核苷酸多型性並無影響。

七、進行〝抗人類免疫缺乏病毒藥品處方使用規範效果分析〞研究結果顯示，病患對於藥物組合的臨床耐受性有所不同，參考 WHO 於 2013 年的治療建議及 2015 訂定的治療目標，Combivir 仍做為國內的治療優先選擇必須配合臨床工作人員注意相關的副作用，而一日兩次的服藥限制對於病患的服藥耐受性是一大考驗，長期的副作用則有待日後更長時間追蹤及更多的病患納入分析。

八、對於 HIV/HBV 合併感染者，無論是曾接受 lamivudine 治療並帶有抗藥性突變的感染者，或是第一線使用抗病毒藥物的病患，都應該依照目前的治療指引選用含有 tenofovir 的抗愛滋病毒藥物組合，以有效抑制 HBV 病毒複製，避免發生慢性 B 型肝炎之相關併發症。未來則需注意追蹤長期使用藥物之可能副作用，及是否可能和其他抗 HBV 藥物一樣發生 HBV 抗藥性突變。

九、探討〝愛滋病毒感染者接種七價結合型肺炎疫苗五年後血清抗體變化〞計畫中我們支持愛滋病毒感染者接種兩劑蛋白結合肺炎鏈球菌疫苗。

十、執行〝愛滋病毒感染者的梅毒發生率及相關因子探討〞我們發現在 2009 年到 2015 年間在台大醫院追蹤的愛滋病毒感染者梅毒發生率持續增加。在臨床照護上，除了持續維持服用愛滋病毒藥物遵囑性以外，持續提供資訊衛教維持安全性行為，也是長期愛滋病毒照護治療的重點。

十一、愛滋病毒感染者在隨著抗病毒藥物的使用之後，感染者存活率提高且生命期也延長，感染者年齡也越來越長，所以慢性病(糖尿病、高血壓、腎臟疾病、骨質疏鬆、代謝症候群)發生的比例也越來越高，長期追蹤型的研究是未來的必然趨勢。本研究為一個世代追蹤型研究，總共追蹤 12 年，其研究的結果可以提供愛滋病照護在照護慢性病時的重要參考指標，提醒臨床照護者，糖尿病在感染者也是需要被關注的議題。

十二、雞尾酒療法時代愛滋感染者發生帶狀疱疹的發生率比起過往已經有降低之情形，但仍比一般非感染者之發生率為高。感染者在開始服用抗病毒藥物前有較低之 CD4 數目，較高之血液愛滋病毒量，以及在服用抗愛滋病毒藥物後有較高幅度之 CD4 數目之增加者，都有較高之機會發生帶狀疱疹。可考慮針對有以上特性之感染者，加強教育以及提醒，讓感染者更瞭解要甚麼情形要及早注意回診，接受適

當之治療，避免發生併發症。

十三、近期國內發生愛滋病毒感染男同志的 HAV 群突發，在此報告完成時，已經有 23 位愛滋病毒感染的男同志被診斷為急性 HAV 感染。根據我們在台大醫院三年來新進開始接受抗愛滋病毒藥物的 852 位男同志的病歷資料調查，我們發現 623 位有檢驗 HAV 抗體，其中抗體呈陽性的比例僅有 15.4%（n=96）。而沒有抗體的易感族群中，僅有 13.1%接種了 HAV 疫苗。這個調查顯示，臨床醫師並沒有依照 ACIP 建議，積極檢測 HAV 抗體，對於抗體陰性的易感族群，HAV 疫苗的接種率很令人擔心的低。接種率過低，可能源於醫師或病患對於急性 HAV 感染的病症大多不嚴重而且也不會有變成慢性的疑慮。其次，HAV 疫苗價格高也是推廣易感受族群全面接種的一大障礙。再此，國際間對於 HAV 疫苗接種的長期追蹤資料也非常缺乏，現有資料無法提供臨床醫療人員或政策制定的公衛專家對於 HAV 疫苗接種建議的參考。我們這個長期追蹤的研究顯示，在持續接受抗愛滋病毒藥物治療情況下，接種三劑的 HAV 疫苗較二劑能維持較持續的保護效果。

十四、著手〝接受全血丙型干擾素釋放分析檢測的 HIV 感染病患之結核病的發生率〞本研究族群 81%為男同志，僅 1.5%為靜脈毒癮使用者，故本研究結論無法推估到其他不同的 HIV 感染族群。再者，因僅一位病患進展成活動性結核病，本研究無法再分析丙型干擾素檢測陽性的 HIV 病毒感染病患進展到活動性結核病的危險因子。此外，追蹤過程中，並不是所有的病患都接受結核病的檢查，而是根據臨床症狀安排結核病的檢查，故可能低估結核病的發生率。最後，隨著年齡的老化和共病的發生，HIV 病毒感染病患罹患結核病的風險可能漸漸增加。最近資料顯示，國內結核病發生率從西元 2005 年的每十萬人口 72 位下降至西元 2012 年的每十萬人口僅 53 位。本研究發現在整體結核病發生率下降的台灣社會中，接受高效能抗病毒藥物治療的 HIV 病毒感染病患發生結核病的風險低。

十五、進行〝接受高效能抗反轉錄病毒治療之後天免疫不全病毒感染患者合併 C 型肝炎病毒感染中接受長效型干擾素合併雷巴威林後之長期病毒預後研究〞，近期國內愛滋病毒感染男同志的 C 型肝炎病毒感染的發生率持續增加。除了行為介入以外，提供定期的追蹤檢測和適當有效的治療，在有效的疫苗開發應用之前，是防治 C 型肝炎擴散傳染的最有效方法。

(六)本計畫對醫藥衛生政策之具體建議

(1)HIV 教育訓練內容及對象多元且豐富未來擬增加感染者之高齡化照護及感染者之移植後照護。

(2)防疫政策上有需要暴露後預防性投藥指引之實施，並針對網路尋求一夜情的族群進行暴露後預防性投藥政策的宣導。

(3)病人要服用以 NNRTI 為基礎的雞尾酒療法，應該在用藥前接受抗藥性基因檢測，以確保藥物治療的效果。

(4)確認國內成立 PK lab 的可行性、監測血中濃度的必要性，ART 血中濃度的結果可提供臨床醫師調整劑量的參考資料，做為國人使用 ART 劑量時的重要依據。例如:使用 EFV600mg 的病患如果體重低於 50 公斤或有服藥副作用者可以建議做藥物濃度檢測，可作為調整其服藥劑量減半或三分之二之參考。

(5)對於低量病毒量患者應該替換成不同機轉的藥物可以改善其病毒被控制的狀況。

(6)對於台灣的愛滋病毒感染者應定期追蹤腹部超音波以便提早發現膽結石及腎結石。

(7)期待新的處方能以實證醫學為基礎，提高台灣愛滋病毒感染者的醫療品質。

(8)對於 HIV/HBV 合併感染者為避免發生慢性 B 型肝炎之相關併發症應選用含有 tenofovir 的抗愛滋病毒藥物組合。

(9)對於 HIV 感染者若要接種蛋白結合肺炎鏈球菌疫苗應接種兩劑效果較佳。

(10)為降低愛滋病毒感染者的梅毒發生率，除了感染者服用愛滋病毒藥物外，應持續對感染者提供安全性行為的衛教資訊。

(11)愛滋病毒感染者慢性病(糖尿病、高血壓、腎臟疾病、骨質疏鬆、代謝症候群)發生的比例越來越高，需要定期且長期追蹤。

(12)服用抗病毒藥物前有較低之 CD4 數目者、較高之血液愛滋病毒量者、 以及在服用抗愛滋病毒藥物後有較高幅度之 CD4 數目之增加者，應給予帶狀疱疹發生率較高的提醒。

(13)在持續接受抗愛滋病毒藥物治療情況下，接種三劑的 HAV 疫苗較二劑能維持較持續的保護效果。

(14) HIV 病毒感染病患應該接受高效能抗病毒藥物治療以降低結核病發生率。

(15)對於國內男同志提供定期的 HCV 追蹤檢測和適當有效的治療，是防治 C 型肝炎擴散傳染的最有效方法。

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附　件

附件一

計畫編號：MOHW104-CDC-C-114-000301

衛生福利部疾病管制署 104 年委託科技研究計畫

醫事人員愛滋病治療照護及全面性防護措施等相關在職訓練課程

年 度 研 究 報 告

執行機構：台大醫院

計畫主持人：洪健清

研究人員：王素華、張乃慈

執行期間：104 年 1 月 1 日至 104 年 12 月 31 日

＊本研究報告僅供參考，不代表本署意見，如對媒體發布研究成果應事先徵求本署同意*

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中文摘要

依據聯合國愛滋病組織（Joint United Nations Programme on HIV/AIDS；UNAIDS）的統計資料顯示，至 2010 年中，全球感染愛滋病人數已達三千三百四十萬人；其中成人約為三千三百萬人；且整體全球感染人數比 2000 年多了 20%；而亞洲感染人數約為四百七十萬人。在亞洲地區，愛滋病的盛行率在性工作者中為 3.4~18%，亞洲地區很多性工作者同時也有使用毒品，所以感染途徑與靜脈毒癮會有重疊的情況。在異性間性行為部分，在亞洲地區有些男性經由性交易感染愛滋病毒之後，又與其性伴侶發生不安全的性行為，讓以往低危險性的異性戀感染率上升。靜脈毒癮者的愛滋病毒盛行率為 6.7~52%，男同性間的愛滋病發生率為 5.2~32.8%。

台灣於民國 75 年年初，第一例本國籍同性戀者死於 AIDS，新感染的個案數即不斷地增加，94 年間國內毒品注射群體爆發一波疫情的成長，幸賴行政院核定「愛滋病減害計畫」以因應毒癮愛滋個案的遽增，94 年 8 月由台北市、台北縣、桃園縣、台南縣進行「毒品病患愛滋減害試辦計畫」；並於 95 年 7 月起擴大辦理清潔針具計畫，全國 23 縣市共計設置清潔針具及愛滋衛教諮商輔導站 427 處；於 95 年 8 月擴大辦理替代療法計畫，全國 22 縣市設置替代療法醫療執行機構，使得毒品注射群體愛滋病盛行率顯著下降，但男同性間的愛滋病毒感染率仍然每年新增比例都超過 15%。再者，台灣與東南亞國家和中國大陸等高感染盛行率地區的交流頻繁，更加速 HIV 感染的擴散。國內累計愛滋病毒感染人數至 99 年 10 月達 2 萬人以上，104 年 9 月底已達 31,425 人（本國籍為 30,432 人）。在愛滋感染者年齡層方面，仍以 20~39 歲為主要族群，但 15~24 歲感染人數卻也逐年增多，大家千萬不能掉以輕心，因此醫療人員在從業的過程中有很大的機會接觸到愛滋病毒感染者，故應該定期接受愛滋病治療照護及全面性防護措施等相關在職訓練。

另一方面為配合疾病管制署關於 HIV 指定醫事機構的指定原則文中第三條規定：醫療人員、藥師及個管師每年需有愛滋病學相關教育學分 8~10 小時，故規劃及執行各科醫事人員針對愛滋病毒感染者照護之相關在職訓練，分北區、南區舉辦「醫事人員愛滋病治療之相關在職訓練課程」，此教育訓練課程有初階及進階等不同的課程內容，參加對象為對於照護愛滋病患有興趣之醫療人員，並與疾病管制署合作宣導及鼓勵其他科別的醫師來參加訓練，籌劃跨科整合的訓練課程，包含感染科、婦產科、兒科、家庭醫學科、精神科、內科、外科、牙科、藥師等醫護人員及社工人員等，同時申請臺灣醫學會、

台灣感染症醫學會、台灣婦產科醫學會、台灣兒科醫學會、台灣愛滋病學會、內科醫學會、台灣家庭醫學會等相關醫學會持續教育學分認證，以提高學員之參加意願，全程參加者結業時並頒發授課證明。

關鍵詞：愛滋病、滋病毒感染、抗病毒藥物、毒品病患愛滋減害計畫、愛滋病減害計畫醫事人員愛滋病治療之相關在職訓練課程

英文摘要

According to the statistics of the Joint United Nations Programme on HIV/AIDS (UNAIDS), 34 million people were living with HIV/AIDS, and among which 23.5 million people are in sub-Saharan Africa. Some 14.8 million people require medical treatment, but only 8 million people have availed of proper treatment. Moreover, the HIV-infected population in the world is 20% higher than that posted in year 2000. In Asia, the HIV-infected population is estimated at 4.7 million people. In the Asian region, the prevalence rate of HIV/AIDS among people plying the sex trade ranges between 3.4% and 18%. Since many sex workers in Asian region are also prohibited substance users, intravenous drug users are likely to come in contact with the infection route of the virus. On the other hand, heterosexual males in the Asian region tend to continue engaging in unsafe sex with their regular sex partners even after being infected with HIV/AIDS through sex trade, thereby resulting in a rise in the infection rate among the previously identified as low-risk heterosexual groups. The HIV/AIDS prevalence rate among the intravenous drug dependents ranges between 6.7% and 52%; whereas, HIV/AIDS prevalence rate among male homosexuals ranges between 5.2 and 32.8%.

In Taiwan, the first reported case of homosexual AIDS sufferer died of the disease in the early period of 1986; thereafter, cases of new HIV infections continue to sprout. In 2005, HIV infection reached an explosive growth rate among the intravenous drug using clusters; consequently, the Executive Yuan ratified the AIDS Harm Reduction Program to cope with the drastic rise of HIV infection among the drug dependent population. In August of 2005, Taipei City, Taipei County, Taoyuan County, and Tainan County organized the AIDS Harm Reduction Program for Drug Dependents; thereafter, a clean syringe campaign was launched in July 2006. There were 427 syringe sterilization and AIDS health information and counseling stations established throughout the 23 cities and counties of Taiwan. In the massive campaign for the use of alternative therapy launched in August 2006, the alternative therapy program implementing facilities established throughout the 22 cities and counties of Taiwan resulted in the significant decrease of AIDS prevalence rate among intravenous drug users; unfortunately, the ratio of new HIV infections reported annually remained to post 15% and above among the male heterosexuals. Furthermore, exchanges between Taiwan and Southeast Asian countries and China having high HIV prevalence rates are quite common, a factor that further hastened the spread of HIV infection. In Taiwan, the total HIV infected

population as of end of Sep. 2015 has reached 31,425 (local nationals comprised 30,432), or an average of 6 persons being infected per day. A more daunting factor is that statistics showed a lowering in the age of HIV-infected persons. Although the 20-39 age group remains to be the primary cluster (74.64%) of people with HIV/AIDS, the ratio of infected people in the 15-24 age group is posting a gradually annual growth, a matter that should not be taken lightly. Since there is a high probability for medical workers to come in contact with people with HIV in their line of duty, it is imperative that they receive regular job training on the matter of AIDS treatment and care and AIDS-related universal precautions.

Moreover, in coordination with Article 3 of the General Designation Principles of the HIV Medical Facilities stipulated by the Centers for Disease Control (CDC) of the Ministry of Health and Welfare, the medical workers, pharmacists, and case managers are required to annually receive the AIDS medicine related education program for 8 to 10 hours; hence, in the future, job training courses on the medical care of people with HIV/AIDS shall be planned and implemented for medical workers of the respect. AIDS treatment related job training courses for medical workers are conducted in the northern and southern regions of Taiwan annually. These education and training courses are classified into beginning and advance courses. Participants are generally medical workers interested in the provision of medical care to people with AIDS. Furthermore, joint campaigns with the CDC will be conducted to disseminate information and to encourage medical practitioners of other fields to join the training program. Cross-disciplinary training programs are being planned for the training of medical workers in the fields of infectious diseases, obstetrics & gynecology, pediatrics, family medicine, psychiatry, internal medicine, surgery, dentistry, and pharmacology, and also social workers. In addition, requests will be forwarded to the medical associations, such as, Formosan Medical Association, Infectious Diseases Society of Taiwan, Taiwan Association of Obstetrics and Gynecology, Taiwan Pediatric Association, Taiwan AIDS Society, Taiwan Society of Internal Medicine, and Taiwan Association of Family Medicine for the accreditation of continuing education credit hours to enhance the participation inclination of medical workers. The course completion certificates are awarded to participants who have satisfactorily completed the entire training courses.

Keywords: HIV, AIDS, HAART, Antiviral drug, Universal precautions.

一、前言

台灣於民國 73 年年底，因一名患有 AIDS 之美籍同性戀者過境台北，一時造成國人的熱烈討論；衛生福利部(前衛生署)遂於 74 年春成立愛滋病防治小組。75 年初第一例本國籍同性戀者死於 AIDS，新感染的個案數即不斷地逐年增加。94 年國內爆發毒癮族群的愛滋病毒感染，當年度的通報感染者人數 3,445 人比 93 年的 1,568 人成長了 119%，為防範藥癮者因共用針具、稀釋液或稀釋容器而感染愛滋病毒，衛生福利部(前衛生署)於 95 年擴大辦理「藥癮愛滋減害計畫」，國內幸賴各相關單位積極參與「藥癮愛滋減害計畫」使得 95 年年底通報人數降為 2,979 人，96 年更降為 1,976 人，但與 92 年以前都只有 3 位數的通報人數相較，仍然有許多防疫上需要努力的地方。再者，台灣與東南亞國家和中國大陸等高感染盛行率地區的交流頻繁，更加速國內 HIV 感染的擴散。目前累計愛滋病毒感染人數至 104 年 9 月底已達 31,425 人（本國籍為 30,432 人）。在愛滋病毒感染者年齡層方面，感染愛滋病毒的年齡層以 25 至 49 歲最多，佔 69.80%，其次為 15 至 24 歲，佔 43.01%，顯示我國愛滋病毒感染者幾乎集中在生產力旺盛的青壯族群，大家千萬不能掉以輕心。

有鑑於醫護人員在從業的過程中有很大的機會接觸到愛滋病毒感染者，所以對於醫護人員的愛滋病毒感染相關知識教育及在職訓練非常重要並有其迫切性。故本計劃規劃及執行各科醫事人員愛滋病治療照護及全面性防護措施等相關在職訓練，每年分別在北區、南區舉辦「醫事人員愛滋病治療照護及全面性防護措施等相關在職訓練課程」，此教育訓練課程有初階及進階等不同的課程內容，參加對象為對於照護愛滋病患有興趣之醫療人員，並與疾病管制署合作宣導及鼓勵其他科別的醫師來參加，籌劃跨科整合的訓練課程，擬包含感染科、婦產科、兒科、家庭醫學科、精神科、內科、外科、藥師等醫護人員及社工人員等，同時申請臺灣醫學會、台灣感染症醫學會、台灣婦產科醫學會、台灣兒科醫學會、台灣愛滋病學會、內科醫學會、台灣家庭醫學會等相關醫學會持續教育學分認證，以提高學員之參加意願，全程參加者結業時並將頒發授課證明。

「愛滋病防治中心」成立 17 年來，業已完成階段性的任務，包括專職人員的訓練招募、醫療設備更新、研究人員的招攬等，但是，儘管感染者的生命存活大為延長之際，新增感染者卻仍持續增加中，這個現象反映出預防工作上的缺失。再者，新的藥物不斷地開發引進台灣，藥物治療的複雜和長期副作用往往不明，長期而言，可能對於感染者產生早發性心血管病變、骨質缺乏、新陳代謝等病變。但是目前國內臨床醫師對於這些

副作用的處理或認知，仍處於渾沌不清的時期；而大量抗病毒藥物的使用，可能導致抗藥性病毒株產生，並進而造成傳播，導致新近感染者治療上的困擾；而病毒株分型的改變，也可能影響到未來疫苗的研發等。在伺機性感染方面，國內仍缺乏長期性、多中心、系統性的研究等，諸如上述問題的解決，都有賴「愛滋病防治中心」繼續運作，並擴大其防治功能，協調各相關醫院的臨床和基礎研究人員，一起研究方能事半功倍。

二、材料與方法

定期分區舉辦「醫事人員愛滋病治療之相關在職訓練課程」，加強醫護人員、臨床醫師對愛滋病的認知。以及每年舉辦一次「全國提昇愛滋病患臨床醫療照顧品質研討會」，及其他科醫療人員之愛滋病研習會，實施方法及進行步驟如下：

（一）初階教育課程，課程內容包括：

1. 台灣愛滋病政策與法令及流行病學介紹
2. HIV 之基因與分子流行病學
3. HIV 感染之檢驗、診斷及臨床表徵
4. 抗愛滋病毒藥物治療指引
5. 愛滋病毒之伺機性感染及治療
6. HIV 門診時之相關醫師衛教
7. 母子垂直感染防治政策及成果
8. 懷孕婦女之抗病毒治療與預防
9. 台灣嬰幼兒愛滋病感染之現況與抗病毒治療
10. HIV 檢驗前後之諮商及家屬衛生教育指導
11. 醫療環境防護措施及人員針扎事件之處理原則
12. HIV/AIDS 之護理照顧

（二）進階教育課程，課程內容包括：

1. HIV/AIDS 抗病毒藥物治療之副作用與交互作用
2. 愛滋病毒之伺機性感染個論
3. HIV/AIDS 之慢性 B 型和 C 型肝炎處置
4. 愛滋病毒感染者之糖尿病
5. 愛滋病毒感染者之心血管疾病與高血脂症之處置

6. 愛滋病毒感染者之糖尿病
7. 愛滋病毒感染者之腸胃疾病
8. 愛滋病毒感染者之腫瘤
9. 愛滋病毒感染者之神經系統疾病
10. 愛滋病毒感染者之性病
11. 愛滋病毒感染者之骨科疾病
12. 抗藥性病毒株之監測及處理
13. HAART 治療失敗病人的處理

（三）靜脈毒癮 HIV 感染者相關之教育訓練課程，課程內容包括：

1. 台灣 HIV 感染及靜脈毒癮 HIV 感染者之流行病學介紹
2. 台灣 HIV 感染及靜脈毒癮 HIV 感染者之分子流行病學介紹
3. HIV/AIDS 毒癮者之治療經驗
4. HIV/AIDS 毒癮者之一般性感染與處置
5. HIV/AIDS 毒癮者之慢性 B 型和 C 型肝炎處置
6. HIV/AIDS 毒癮者之精神疾病與處置
7. HIV/AIDS 毒癮者之護理照護經驗
8. 「減少傷害 Harm Reduction」之相關工作坊及訓練課程

（四）參加對象：對於照護愛滋病患有興趣之醫事人員，包含感染科、婦產科、兒科、家庭醫學科、精神科、內科、外科、藥師等醫護人員及社工人員。

（五）將申請臺灣醫學會、台灣感染症醫學會、台灣婦產科醫學會、台灣兒科醫學會、台灣愛滋病學會、內科醫學會、台灣家庭醫學會等相關醫學會持續教育學分認證，以提高學員之參加意願。

（六）全程參加者結業時頒發授課證明。

（七）進行課程評量，瞭解學員之需求及意見，以做為辦理下屆訓練課程之參考。

三、結果

愛滋病毒感染相關的知識日新月異，新藥物與治療的研發蓬勃發展，面對這些醫療新知的獲取，對照護愛滋病患的醫事人員而言格外重要且迫切，故愛滋病防治中心扮演傳播及教育國內治療與防治相關知識的角色，積極且定期規劃及執行各科醫事人員「愛

滋病治療照護及全面性防護措施等相關在職訓練」課程。

(1)104 年 3 月 14 日於台北舉辦「2015 醫療人員 Post-CROI 研討會」邀請參與 2015 CROI 會議的愛滋病毒感染治療學者專家，在會後整理會議資料，有系統地介紹給國內的醫師和個管師。衛生署指定醫療院所照護愛滋病毒感染者之專責醫師及個管師共 35 位參與。研討會內容：

時　　段	講　　題	講　　者	主 持 人
13:30~13:50	Registration		
13:50~14:00	Opening		林錫勳 醫師
14:00~14:40	HIV virology	張淑媛 教授	林錫勳 醫師
14:40~15:20	Newer ART and trials	顧文瑋 醫師	王永衛 醫師
15:20~16:00	Metabolic complications (epidemiology and mechanism)	楊家瑞 醫師	林育蕙 醫師
16:00~16:20	Coffee Break	All	
16:20~17:00	Hepatitis Coinfection	李官燁 醫師	洪健清 醫師
17:00~17:40	Prophylaxis against HIV	李佳雯 醫師	柯乃熒 教授
17:40~18:20	Opportunistic illnesses (infection, malignancy and others)	蔡茂松 醫師	何茂旺 醫師
18:20~18:30	Discussion & Closing	All	林錫勳 醫師

(2) 104 年 3 月 28 日舉辦「愛滋病毒跨院教育與研究」共集結國內臨床醫療及研究學者專家共 35 位一起討論有關「臨床照護教育及研究平台」，有兩項討論及 17 點回饋。

(3) 104 年 4 月 29 日舉辦「台灣急性愛滋病毒感染研究討論會」國內臨床醫療及研究學者專家共 17 位出席，有 6 項討論及 10 點回饋。

Time	Topic	Speaker	Moderator
18:00-18:25	Registration		
18:25-18:30	Opening		林錫勳 醫師

18:30-19:00	Literature review of acute HIV infection	蔡茂松醫師	洪健清 醫師
19:00-19:30	Proposed research on acute HIV infection in Taiwan	林德宇醫師	王甯祺 醫師
19:30-20:25	Discussion		林錫勳 醫師
20:25-20:30	Closing and plan for the next meeting		林錫勳 醫師

(4) 為配合衛生福利部疾病管制署對於指定醫療院所照護 HIV/AIDS 醫護人員之在職訓練要求，104 年 6 月 7 日(日)於國立成功大學醫學院第三講堂舉辦「藥師愛滋病治療專業能力教育訓練課程」，參加對象：任職後天免疫缺乏症候群指定醫療院所的藥師，參與減害計畫指定醫院或藥房之藥師，其他對於愛滋病治療有興趣的藥師。統計參會者共有 112 位藥師。課後評量統計表詳見附件一~1。課程內容如下：

時　　間	題　　目	演講者	主持人
08:50~09:20	Registration		
09:20~09:30	Welcome address		林錫勳 醫師 高雅慧 教授
09:30~10:20 10:20~10:50	愛滋病毒感染的藥物治療 Presentation of Cases-1	黃俊凱 醫師	林錫勳 醫師
10:50~11:10	Coffee Break		
11:10~12:00 12:00~12:30	特殊族群之愛滋病毒感染治療 Presentation of Cases-2	李佳雯 醫師	柯乃熒 教授
12:30~13:40	Lunch		
13:40~14:30 14:30~15:00	如何處理愛滋病毒感染治療效果不佳的病人 Presentation of Cases-3	蔡宏津 醫師	陳垚生 醫師
15:00~15:20	Coffee Break		
15:20~16:10 16:10~16:40	愛滋病毒感染藥物治療的交互作用 Presentation of Cases-4	林文亮 藥師	李南瑤 醫師
16:40~17:10	Discussion & Closing		李南瑤 醫師

(5) 7 月 11、12 日特別開立「2015 全國愛滋病毒感染個案管理師教育訓練研習班」，共有國內指定醫師院所現職之臨床愛滋病毒感染個案管理師 57 位完成課程。課程內容如下：

7/11 日(六)	題 目		演講者	主持人
09:40-10:00	報 到			
10:00-10:10	歡 迎			林錫勳 理事長
10:10-10:50	HAART 藥物副作用之評估與處理		洪健清 醫 師	林錫勳 理事長
10:50-11:30	女性(懷孕)感染者諮商會談技巧		賴怡因 個管師	林錫勳 理事長
11:30-12:10	懷孕婦女愛滋寶寶照護		施鐘卿 個管師	林錫勳 理事長
12:10-12:30	討 論			林錫勳 理事長
12:30-13:30	午 餐			
13:30-14:00	個案管理實務綜論		柯乃熒 秘書長	柯乃熒 秘書長
14:00-17:30	個案管理實務分享		柯乃熒 秘書長	柯乃熒 秘書長
	分組討論	服藥遵從性評估(Transgender 併使用娛樂性藥物成癮藥物者)	A 組(莊苹主任)	
		危險性行為的解決策略(併使用娛樂性藥物成癮藥物者)	B 組(謝佳吟個管師)	
		疾病告知(相異異性戀伴侶，懷孕 10 週)	C 組(白芸慧個管師)	
		未成年照護(17 歲 MSM)	D 組(巫沛瑩個管師)	
17:30-18:00	討論與心得分享			柯乃熒 秘書長
7/12 日(日)	題 目		演講者	主持人
09:00-09:40	性病評估與處置		王建淳 醫師	王永衛 常務理事
09:40-10:20	(即將)旅外感染者照護		李欣純 醫師	王永衛 常務理事
10:20-10:40	茶 敘			
10:40-11:20	愛滋修法之重點		林宜慧 秘書長	林育蕙 監事
11:20-12:00	感染者安置與資源轉介		徐森杰 秘書長	林育蕙 監事
12:00-12:30	綜合討論			王永衛常務理事 林育蕙 監事

(6) 為提昇國內感染症專科醫師愛滋病治療專業能力及參與力，繼續與台灣感染症醫學會、台灣愛滋病學會，針對有興趣從事愛滋病治療的年輕感染症專科醫師開立「感染症專科醫師愛滋病治療專業能力教育訓練研習班」。參加對象為感染症專科醫師訓練醫院之第 4、5 年住院醫師，將於 104 年 9 月 12~13 日共 1.5 天，洽借公務人力發展中心福華國際文教會館舉辦，計有 52 位感染科住院醫師參加。本次研習班有針對參與學員個人的收穫及講師進行課程的表現，請學員提供意見以利主辦單位列入評估及作為將來改進的參考，故請學員填寫課程評量表。課後評量統計表詳見附件一~3。本年度課程內容如下：

Sep. 12 (六)	Topic	講　　師	主持人
08:30-09:00	Registration		
09:00-09:10	Opening		顏慕庸 院長
09:10-09:50	Update on HIV virology: diagnosis and monitoring	張淑媛 教授	顏慕庸 院長
09:50-10:40	Introduction to combination antiretroviral therapy	吳冠陞 醫師	顏慕庸 院長
10:40-11:00	Break		
11:00-11:50	Management of opportunistic infections and malignancy: gastrointestinal and others (1)	蔡茂松 醫師	王永衛 醫師
11:50-12:40	Management and prophylaxis of opportunistic infections and malignancy: respiratory and nervous systems (1)	鄭健禹 醫師	王永衛 醫師
12:40-14:00	Lunch		
14:00-14:50	Hepatitis co-infections: epidemiology, prevention and management	顧文瑋 醫師	王甯祺 醫師
14:50-15:40	Complications of combination antiretroviral therapy	黃俊凱 醫師	王甯祺 醫師
15:40-16:00	Break		
16:00-16:50	Transmitted drug resistance: update and management	蔡宏津 醫師	莊銀清 理事長
16:50-17:40	Case demonstration (1) - respiratory and nervous system	盛望徽 醫師	莊銀清 理事長
17:50-18:40	Case demonstration (2) - gastrointestinal and others	李佳雯 醫師	林錫勳 理事長
18:40-19:00	Q and A		林錫勳 理事長

Sep. 13 (日)	Topic	講　　師	主持人
08:30-09:20	Management of pediatric patients with HIV infection: from birth to teenage	呂俊毅 醫師	謝思民 醫師
09:20-10:10	Management of serodiscordant couple preparing for pregnancy and HIV-infected women with pregnancy	鄭舒倖 醫師	謝思民 醫師
10:10-10:30	Break		
10:30-11:20	Management of common sexually transmitted infections	王建淳 醫師	林育蕙 醫師
11:20-12:10	Update of management of Pre- and post-exposure prophylaxis in occupation and non-occupational settings	楊家瑞 醫師	林育蕙 醫師

(7) 104 年 9 月 4~5 日於台南與成大醫院共同舉辦「104 年度台灣多中心愛滋病學術研討會 Taiwan HIV/AIDS Research Meeting」會中並邀請研究梅毒之比利時專家 Dr. Chris Kenyon 專題演講，並與國內專家學者共同討論，計有 52 位專家學者參與。

Sep.4	Topic	Speaker	Moderator
6:30-6:40	Opening	Dr. Chuang YC	
6:40-7:20	Host factors and HIV progression	Prof. Huang LM	Dr. Chuang YC
7:20-8:00	Current status of antiretroviral resistance, including pre-treatment resistance to integrase inhibitors, in Taiwan	Prof. Chang SY	Dr. Chuang YC
8:00-8:20	Transmitted drug resistance of HIV-1 strains among individuals attending voluntary counseling and testing in Taiwan	Dr. Lai CC	Dr. Ko WC Dr. Hung CC
8:20-8:50	1). National trend and characteristics of acute hepatitis C among HIV-infected individuals: a case-control study-Taiwan, 2001-2014; AND 2). Microbiologic, clinical, and epidemiologic investigations of recent outbreak of shigellosis among MSM in Taiwan	Dr. Lo YC	Dr. Ko WC Dr. Hung CC
8:50-9:10	Discussion		
9:10-9:15	Closing remarks		Dr. Ko WC

Sep.5	Topic	Speaker	Moderator
8:50-9:00	Opening	Dr. Ko WC	
9:00-9:40	Features of the Treponema pallidum proteome and Homo sapiens behaviour that explain the persistence of syphilis	Dr. Chris Kenyon (ITM, Antwerp, Belgium)	Dr. Lin HH Dr. Cheng SH
9:40-10:00	Prior condyloma and syphilis diagnosis among HIV-infected persons: a nationwide population-based study in Taiwan, 2000-2010	Dr. Carol Strong	Dr. Lin HH Dr. Cheng SH
10:00-10:20	Unexpectedly high prevalence of Treponema pallidum infection in the oral cavity of HIV-infected patients with early syphilis who reported unprotected oral sex practices	Dr. Yang CJ	Dr. Lin HH Dr. Cheng SH
10:20-10:40	Discussion		
10:40-10:50	Coffee Break		
10:50-11:10	HIV and HBV infection: KVGH experience-sharing	Dr. Tseng YT	Dr. Tsai HC Dr. Sheng WH
11:10-11:30	Virological response to tenofovir in HIV-infected patients with lamivudine-resistant hepatitis B virus co-infection in an area hyperendemic for hepatitis B virus infection	Dr. Lee KY	Dr. Tsai HC Dr. Sheng WH
11:30-11:50	Trends of hepatocellular carcinoma in HIV-infected patients: an analysis using NHI databases	Dr. Lin SP	Dr. Tsai HC Dr. Sheng WH
11:50-12:10	Discussion		
12:10-13:20	Lunch		
13:20-13:40	Toll-like receptors gene variants in HIV-infected patients with nevirapine-related skin rash	Dr. Hung YP	Dr. Lee SS Dr. Ho MW
13:40-14:00	Therapeutic drug monitoring of nevirapine (NVP) in HIV-infected patients switching from NVP IR to NVP XR	Dr. Chang HM	Dr. Lee SS Dr. Ho MW
14:00-14:20	Fibroblast growth factor 23 correlates with hypophosphatemia and bone mineral density change in HIV-infected patients receiving antiretroviral therapy	Dr. Lin DY	Dr. Lee SS Dr. Ho MW

14:20-14:40	Discussion		
14:40-14:50	Coffee break		
14:50-15:10	Long term renal outcome of tenofovir and boosted PI among HIV-infected patients in Taiwan	Dr. Chen TC	Dr. Tang HJ Dr. Wang NC
15:10-15:30	Tenofovir-associated nephrotoxicity and hypophosphatemia among HIV-infected patients: a single-center cohort study	Dr. Cheng CY	Dr. Tang HJ Dr. Wang NC
15:30-15:50	Long-term immune responses to one or two doses of heptavalent conjugated pneumococcal vaccine (PCV7) in HIV-infected adults in the era of combination antiretroviral therapy	Dr. Cheng A	Dr. Tang HJ Dr. Wang NC
15:50-16:10	Discussion		
16:10-16:30	Association between the use of efavirenz and the occurrence of depressive disorders among HIV-treated patients	Dr. Lee CW	Dr. Ko NY Dr. Lau CE
16:30-16:50	HIV medication readiness, adherence and viral load suppression among HIV-infected patients	Miss Lai YY, Miss Hsieh CY, Miss Lin CD	Dr. Ko NY Dr. Lau CE
16:50-17:10	1). Comparison of sleep disorder among patients with HIV, cancer and general population; OR 2). Statin and cardiovascular events among people living with HIV	Dr. Chen YC Dr. Ou HT	Dr. Ko NY Dr. Lau CE
17:10-17:30	Discussion		
17:30-17:40	Closing remarks		Dr. Chen YH

(8) 9/7 日在台北舉辦「Symposium of Management of Sexually Transmitted Diseases」邀請研究梅毒之比利時專家 Dr. Chris Kenyon 專題演講，有35 位醫療人員參與。

Time	Topic	Speaker	Moderator
18:20-18:30	Opening	顏慕庸 醫師	
18:30-19:10	Current status of STDs in Taiwan	黃士澤 醫師	王甯祺 醫師
19:10-19:50	Current status of antimicrobial resistance of Neisseria gonorrhoeae in Taiwan	王建淳 醫師	王永衛 醫師
197:50-20:30	Treatment of early syphilis in HIV-infected patients in Taiwan	楊家瑞 醫師	鄭舒倖 醫師
20:30-21:10	Certain attributes of the sexual ecosystem of high-risk MSM have resulted in an altered microbiome with an enhanced propensity to generate and transmit antibiotic resistance	Dr. Chris Kenyon	洪健清 醫師
21:10-21:20	Panel discussion	顏慕庸 醫師	

(9) 8/22 日 HIV Resistance(抗藥性)Workshop 基礎班有 44 位醫療人員參與，11/17 日的 HIV Resistance(抗藥性)Workshop 進階班預計有 40 位醫療人員參與。HIV 的治療需考慮許多因素，而抗藥性是治療中相當重要的一環。為了讓國內醫師更熟悉 HIV 及抗藥性的重要性，特別設計了一系列的課程，期望醫護人員於課程結束後，能在治療上更加得心應手，嘉惠更多病患，故舉辦此教育訓練課程。

8/22 日	Topic	Speaker	Moderator
09:00-09:10	課前筆試 (不記名)		
09:10-09:20	Opening	林錫勳 主任 義大醫院	
09:20-10:20	Introduction of HIV treatment and regimen	蔡宏津 醫師 高雄榮總	林錫勳 主任 義大醫院
10:20-10:40	Break		
10:40-11:30	Overview of HIV disease progress, infection, monitoring, and treatment	黃俊凱 醫師 義大醫院	林錫勳 主任 義大醫院
11:30-12:00	Discussion	黃俊凱、林錫勳、蔡宏津	
12:00-13:00	Lunch 午餐時間		
13:00-14:00	Introduction of transmitted drug resistance (TDR) and role of TDR in HIV treatment	張淑媛 教授 台大醫院	洪健清 醫師 台大醫院
14:00-14:10	Break		
14:10-15:50	Application of TDR in clinical practice / case sharing	洪健清 醫師 台大醫院 & 蔡宏津 醫師 高雄榮總	林錫勳 主任 義大醫院
15:50-16:20	Q&A / Discussion	張淑媛、洪健清、蔡宏津、林錫勳	
16:20-16:50	筆試題目解說	張淑媛、洪健清、蔡宏津、黃俊凱	
16:50-17:00	Closing	林錫勳 主任 義大醫院	

11/17 日	Topic	Speaker	Moderator
9:30-10:00	報到		
10:00-10:10	Opening	林錫勳 主任 義大醫院	
10:10-10:20	課前測驗	All	
10:20-11:00	Resistance update	蔡宏津 醫師 高雄榮總	林錫勳 主任 義大醫院
11:00-11:50	Resistance demo	張淑媛 教授 台大醫院	林錫勳 主任 義大醫院
11:50-12:00	Q&A / Discussion	All	
12:00-13:00	Lunch 午餐時間		
13:00-13:50	Case sharing I (lecture 30 min + Q&A 20 min)	楊家瑞 醫師 亞東醫院	洪健清 醫師 台大醫院
13:50-14:40	Case sharing II (lecture 30 min + Q&A 20 min)	黃崧溪 醫師 台大醫院	洪健清 醫師 台大醫院
14:40-15:00	Break		

15:00-15:50	Case sharing III (lecture 30 min + Q&A 20 min)	李育霖 醫師 彰基	洪健清 醫師 台大醫院
15:50-16:40	Case sharing IV (lecture 30 min + Q&A 20 min)	林德宇 醫師 台北三軍總醫院	洪健清 醫師 台大醫院
16:40-17:00	測驗題目解說	All	
17:00	Closing	林錫勳 主任 義大醫院	

(10) 10/31~11/1 日邀請英國具有 HIV 病患器官移植經驗的專家 Dr. Frank Post 專題演講，在台中舉辦「Post-IAS & EACS new strategy update & HIV transplantation issues update」有47 位醫療人員參與。課程內容如下:

Date	Time	Topic	Speaker	Moderator
2015/10/31	14:30－14: 40	Opening	林錫勳理事長	
	14:40－15:20	Prophylaxis against HIV	成大李佳雯	北榮王永衛
	15:20－16:10	ART update & complication issues	亞東楊家瑞	高榮蔡宏津
	16:10－16:20	Break		
	16:20－17:10	Opportunistic infection	彰基張志演	彰基劉尊榮
	17:10－18:00	HAART in Taiwan: from sciences to policy	台大羅一鈞	台大謝思民
	18:00－19:30	IAS & EACS updating	Dr. Frank Post	台大洪健清
	19:30－20:00	Closing & Discussion	台大洪健清	
2015/11/01	09:00－09:10	Opening	林錫勳理事長	
	09:10－10:10	Issues of kidney transplantation among HIV infected patients	Dr. Frank Post	成大李伯璋
	10:10－10:50	Willingness to donate organ among HIV---infected patients	台大孫幸筠	中榮林育蕙
	10:50－11:10	Discussion on Transplantation issue	主持人：台大洪健清 與談人：Dr. Frank Post /成大李伯璋/台大孫幸筠/中榮林育蕙	
	11:10－11:20	Break		
	11:20－12:10	Hepatitis co---infection	北榮顧文瑋	部桃鄭舒倖
	12:10－12:40	Closing & Discussion	台大洪健清	

(11)本中心延續以往每週一次的愛滋病研討會，固定于每週二早上在綜合病房研討室舉行，本年度聘請了各方面的專家來進行全方位的研討，其內容包括有臨床醫學、病毒學、免疫學、流行病學、護理學、精神科醫學、個案研究、研究成果發表及新抗病毒藥物之介紹等；參加成員亦日益踴躍，包括有各科各級醫師、護理人員、檢驗人員、助理人員、社工人員、各基礎學科教師，踴躍參與，以期大家能各憑專業集思廣益。本年度 1~11 月份已進行 26 場。其題目及演講者如附件一~4。

四、結論與建議

本計畫已達成之目標、結論與建議如下：

一、本計畫在104 年已主辦3 場次大型在職教育訓練課程及研討會、2 場次 workshop、3 場次中小型研討會，各場次皆有醫療人員約 47~112 人次參與，對國內愛滋病防治之醫療教育貢獻良多。

二、為提昇國內 HIV/AIDS 臨床及學術研究，104 年度舉辦 4 場「台灣地區 HIV/AIDS 多中心研究合作平台討論會」。每一場次約有醫師及研究人員 35~52 名參加，會中互相討論做成許多共識與結論。明年度擬繼續舉辦。

三、每週一次的愛滋病防治中心研討會，本年度繼續聘請各界的專家來進行全方位的研討，內容包括臨床醫學、藥學、病毒學、免疫學、流行病學、護理學、精神科醫學、個案研究、研究成果發表及新抗病毒藥物之介紹等；以後將加強個案討論，以期大家能各憑專業集思廣益互相交流。明年度擬繼續定期舉辦。

四、105 年度擬定期於北區及中區舉辦 HIV 感染者病例個案討論會，由各區之指定醫療院所輪流提出各院診療之有意義及特殊的病例，和與會者一起討論。

五、明年度希望加強定期舉辦各次專科及藥師相關愛滋病毒感染的繼續教育研討會，藉以增進其他次專科及藥師對於愛滋病毒感染的認知。

六、HIV 教育訓練內容及對象多元且豐富未來擬增加感染者之高齡化照護及感染者之移植後照護。

五、參考文獻

1. 92 年 2 月 22~23 日屏東舉辦「全國提昇愛滋病患臨床醫療照顧品質研討會」。
2. 92 年 8 月 16 日台北、9/13 日台中、9/20 日高雄，各一場「抗 HIV 藥物繼續教育課程 2003」。

3. 92 年 11 月 1~2 日台北舉辦「2003 Updated Management of HIV Infections in Taiwan-Working to Success」。
4. 93 年 4 月 10 日台南舉辦「2004 全國提昇愛滋病臨床醫療照顧品質研討會」。
5. 93 年 4 月 17 日北區舉辦教育訓練課程—台灣地區愛滋病診斷與治療之最新進展。
6. 93 年 10 月 16 日台北舉辦「2004 愛滋病毒感染者之相關伺機性感染研討會」。
7. 93 年 11 月 20 日舉辦「校園愛滋病防治教育研習會（北區）」。
8. 93 年 12 月 18 日台大醫院第七講堂舉辦「2004 HIV/AIDS 醫護人員的新挑戰研討會」。
9. 94 年 3 月 5 日舉辦「2005 全國提昇愛滋病患臨床醫療照顧品質研討會」。
10. 94 年 6 月 6~10 日舉辦「指定藥癮治療業務醫療機構之醫事人員照護毒癮愛滋個案藥癮戒治和愛滋病治療專業能力之培訓和教育訓練案」課程。
11. 94 年 8 月 13 日假台南成大舉辦「2005 HIV/AIDS 醫護人員的新挑戰研討會」。
12. 94 年 8 月 15 日在台大醫院景福館舉辦「改變藥癮行為的階段性治療模式工作坊」。
13. 95 年 4 月 29 日在台大醫學院第 101 講堂，舉辦「2006 全國提昇愛滋病患臨床醫療照顧　　品質研討會」。
14. 95 年 6 月 8~9 日美國臨床心理師 Patt Denning 博士舉辦為期 2 天之「減少傷害心理治療 模式訓練工作坊」。
15. 95 年 7 月 8 日在台大公衛學院 101 講堂舉辦「醫療人員愛滋病治療專業能力初階教育訓練課程」。
16. 95 年 11 月 18 日在衛生署彰化醫院合辦「愛滋病感染管制教育訓練課程」。
17. 95 年 11 月 25 日在台大醫院國際會議舉辦「醫療人員愛滋病治療專業能力進階教育訓練課程」。
18. 95 年 12 月 11 日財團法人羅東博愛醫院舉辦「台灣地區愛滋病診斷與治療之最新進展」研討會。
19. 96 年 4 月 14~15 日於羅東舉辦「醫療人員愛滋病治療專業能力進階教育訓練課程」。
20. 96 年 6 月 2 日於台北舉辦「Update management of HIV: Workshop for HIV co-infection disease」。
21. 96 年 6 月 23 日台大醫院國際會議舉辦「愛滋病毒感染治療藥物新進展介紹」。
22. 96 年 9 月 29 日在台北市舉辦本年度的 HIV/AIDS Workshop for Drug Resistance and Treatment Options。

23. 96 年 10 月 13、14、20、21 日大同大學尚志教育館一樓 106 會議室及 103 電腦教室舉行「感染症專科醫師藥品臨床研究設計及執行研習班」。
24. 96 年 11 月 3 日台北市徐州路 2 號台大醫院國際會議中心 101 講堂舉辦「愛滋病毒感染者之新陳代謝相關問題研討會」。
25. 96 年 12 月 15 日台北市仁愛路一段 1 號台大醫學院 101 講堂舉辦「醫療人員 HIV 體液暴露後之諮詢、檢驗、診斷及治療相關研討會」教育訓練課程。
26. 97 年 5 月 3 日高雄市左營區蓮潭國際文教中心講堂舉辦「醫療人員愛滋病治療專業能力進階教育訓練課程」。
27. 97 年 6 月 28 日台南成大醫院舉辦「醫療人員 HIV 體液暴露後之諮詢、檢驗、診斷及治療相關研討會」。
28. 97 年 9 月 25 日台中地方法院檢察署第二辦公大樓六樓會議室舉辦「監所受刑人回歸社區之愛滋病/性病/肝炎防治工作坊」。
29. 97 年 10 月 4 日羅東博愛醫院 5 樓大禮堂舉辦「醫療人員愛滋病治療專業能力初階教育訓練課程」。
30. 97 年 11 月 1 日台大醫學院 101 講堂舉辦「醫療人員愛滋病治療專業能力進階教育訓練課程」邀請美國 Buffalo University 蕭秋彬博士主講「HIV 感染者之治療新展望」。
31. 97 年 12 月 7 日署立台北醫院 8 樓大禮堂舉辦「牙科醫療人員愛滋病治療專業能力初階教育訓練課程」。
32. 98 年 3 月 7 日台大醫院國際會議中心 401 室舉辦「醫療人員愛滋病治療專業能力進階教育訓練課程」。
33. 98 年 5 月 3 日台中裕元花園酒店舉辦「監所及看守所毒癮愛滋病毒感染受刑人相關問題座談會」。
34. 98 年 6 月 20 日台大醫學院 101 講堂、104 講堂舉辦「醫療人員愛滋病治療專業能力初階教育訓練課程」。
35. 98 年 8 月 6 日台灣大學醫學院 102 講堂舉辦「98 年北部地區 HIV 篩檢前後諮詢人員訓練」。
36. 98 年 8 月 15 日台灣大學醫學院 102 講堂舉辦「藥師愛滋病治療專業能力教育訓練課程」。
37. 98 年 9 月 5 日 桃園縣南方莊園舉辦「醫療人員愛滋病治療專業能力進階教育訓練課

程」。

38. 98 年 9 月 19 日台南市遠東國際大飯店舉辦「愛滋個案管理計畫相關醫師及個管師座談會」。
39. 98 年 10 月 31 日舉辦「愛滋病個案管理師訓練」(北區 10/31~11/1 日)、(中區 11/20~21 日)、(南區 11/6~7 日) 」。
40. 99 年 3 月 6~7 日交通部國際會議中心 3 樓講堂舉辦北區「愛滋病治療專業能力教育訓練課程」。
41. 99 年 3 月 13~14 日彰化基督教醫院教學研究大樓 12 樓蘭大衛國際會議廳舉辦中區「愛滋病治療專業能力教育訓練課程」。
42. 99 年 3 月 20~21 日高雄榮民總醫院第二會議室舉辦南區「愛滋病治療專業能力教育訓練課程」。
43. 99 年 5 月 16 日台中亞緻大飯店會議廳舉辦「愛滋病毒匿名篩檢計畫座談會」。
44. 99 年 5 月 30 日台中日華金典酒店舉辦「2010 提昇愛滋病患臨床醫療照顧品質研討會—HIV 合併 HBV 之相關問題」。
45. 99 年 8 月 17 日及 8 月 20 日 Conference of Immune Reconstitution Disease (高雄場及台北場)。
46. 99 年 8 月 21~22 日台北縣深坑福容大飯店與台灣感染症醫學會、台灣愛滋病學會合辦「感染症專科醫師愛滋病治療專業能力教育訓練研習班」。
47. 99 年 11 月 20 日舉辦「2010 年全國 HIV 及 TB 治療指引研討會」。
48. 99 年 12 月 5 日署立台北醫院 8 樓大禮堂舉辦「牙科醫療人員愛滋病治療專業能力初階教育訓練課程」。
49. 100 年 3 月 12 日台大醫學院 101 講堂舉辦「2011 醫療人員 HIV/AIDS 治療座談會」。
50. 100 年 5 月 22 日台中亞緻大飯店會議廳舉辦「2011 愛滋病毒匿名篩檢及個案管理計畫座談會」。
51. 100 年 6 月 11 日高雄市蓮潭會館 1 樓講堂協辦「愛滋病最新進展國際研討會」。
52. 100 年 7 月 8 日舉辦「2011 年度愛滋病防治中心期中研究成果討論會」。
53. 100 年 9 月 17~18 日公務人力發展中心福華國際文教會館舉辦「感染症專科醫師愛滋病治療專業能力教育訓練研習班」。
54. 100 年 12 月 10 日台大醫學院 101 講堂召開「2011 醫療人員 HIV/AIDS 治療座談會」。

55. 100 年 12 月 10 日召開「台灣地區 HIV/AIDS 多中心研究合作平台討論會」。
56. 100 年 12 月 15 日舉辦「2011 年度愛滋病防治中心研究成果討論會」。
57. 101 年 2 月 17 日舉辦「101 年度台灣地區 HIV/AIDS 多中心研究合作平台討論會 I」。
58. 101 年 5 月 26 日國立成功大學醫學院護理學系 3 樓 309 教室舉辦「101 年度進階愛滋個管工作坊」。
59. 101 年 6 月 16 日台大國際會議中心 401 講堂舉辦「藥師愛滋病治療專業能力教育訓練課程」。
60. 101 年 9 月 15~16 日舉辦「感染症專科醫師愛滋病治療專業能力教育訓練研習班」。
61. 101 年 12 月 8 日台大醫學院 102 講堂再度召開「2012 醫療人員 HIV/AIDS 治療座談會」。
62. 102 年 1 月 12 日(六)舉辦「102 年度台灣地區 HIV/AIDS 多中心研究合作平台—梅毒研究討論會 I」。
63. 102 年 5 月 5 日(星期日)於高雄榮民總醫院會議中心第二會議室舉辦「藥師愛滋病治療專業能力教育訓練課程」。
64. 102 年 5 月 31 日(五)舉辦「102 年度台灣地區 HIV/AIDS 多中心研究合作平台—愛滋病毒抗藥性監測計劃討論會」。
65. 102 年 6 月 15 日(六)於台大國際會議中心 4 樓 402 室舉辦「Streptococcus pneumoniae infection and pneumococcal vaccination among HIV-infected adults in the era of combination antiretroviral therapy」研討會。
66. 102 年 9 月 28~29 日公務人力發展中心福華國際文教會館舉辦「感染症專科醫師愛滋病治療專業能力教育訓練研習班」。
67. 103 年 5 月 3 日(星期六)於台大國際會議中心 402 講堂舉辦「藥師愛滋病治療專業能力教育訓練課程」。
68. 103 年 8 月 30、31 日 (1.5 日)於台北舉辦「103 年度台灣地區 HIV/AIDS 多中心研究合作平台學術研討會」。
69. 103 年 7 月 12~13 日(1.5 日)於公務人力發展中心福華國際文教會「HIV 個案管理師教育訓練研習班」。
70. 103 年 9 月 20~21 日(1.5 日)於公務人力發展中心福華國際文教會館舉辦「感染症專科醫師愛滋病治療專業能力教育訓練研習班」。

附件一~1

104年度藥師愛滋病治療專業能力教育訓練課程

課程評量統計表(日期:104年6月7日)

	非常滿意 5	滿意 4	普通 3	不滿意 2	差 1
課程					
1. 時間安排控制	45.0%	54.0%	1.0%		
2. 講義教材	45.0%	54.0%	1.0%		
3. 課程內容的結構性	43.0%	57.0%	0.0%		
場地餐食					
1. 場地設施、空間的滿意程度	41.0%	57.0%	2.0%		
2. 您對餐點(量與質)的滿意程度	42.0%	50.0%	8.0%		
講題內容對 您目前工作的幫助程度					
講題一：愛滋病毒感染的藥物治療	44.0%	55.0%	1.0%		
Presentation of Cases-1	44.0%	55.0%	1.0%		
講題二：特殊族群之愛滋病毒感染治療	40.0%	55.0%	5.0%		
Presentation of Cases-2	42.0%	54.0%	4.0%		
講題三：如何處理愛滋病毒感染治療效果不佳的病人	45.0%	50.0%	5.0%		
Presentation of Cases-3	45.0%	53.0%	2.0%		
講題四：愛滋病毒感染藥物治療的交互作用	45.0%	54.0%	1.0%		
Presentation of Cases-4	45.0%	54.0%	1.0%.		
總評					
1. 您對本課程整體收獲的滿意程度	58%	42%			

2. 本次課程需改進的地方：

教材講義部份：

勿用黑底白字很難做註記、希望可以用白底黑字黑底浪費也不易做筆記、 good(x2) 、希望ppt不要有黑底、投影片底色黑的有時不好閱讀寫筆記(x4)、投影片一頁2張有點浪費紙不環保

內容結構部份：

good(x2)!

講師教學部份：

很認真準備資料及教學、皆風趣且不贅述案例符合執業需求、時間控制恰當

其他建議事項：

內容豐富但一時難完整吸收可否拍成DVD有想複習者或常用者可重覆學習、課程順序也許可以調整先做藥物介紹再做藥物治療的了解能有助學習、課程安排時間有些緊湊感覺講師還有很多想講想分享的、這次上課的時間安排太緊湊、可以早點上課

3. 下一次課程您最希望聽到的議題為：

個管師如何與病人衛教溝通、治療選擇、HIV的藥物治療、抗藥性處理原則、關於愛滋寶寶的防治、特殊族群之HIV用藥、藥物resistant判讀何時某些drug resistant但又同時上某些drug效果理由為何?國內相關study結果?HIV藥物PT衛教、都可、伺機性感染

4. 下一次課程您最希望聽到那一位講師的演講：

楊家瑞(1)、謝思民(1)、李官燁(1)、洪健清(1)、林文亮(10)、黃俊凱(3) 、李佳雯(5) 、蔡宏津(3) 、都好、林文亮藥師真的很厲害!

附件一~2

愛滋病毒感染個案管理師教育訓練研習班

104年度課程評量統計表(104/7/12)

	非常滿意% 10 9	滿意% 8 7	普通% 6 5	不滿意% 4 3	差% 2 1
課程					
1.時間安排控制	50.8	44.0	5.2	0	0
2.講義教材	67.8	30.5	1.7	0	0
3.研習班內容的結構性	67.8	32.2	0	0	0
場地餐宿					
1.場地設施、空間的滿意程度	61.0	28.8	10.2	0	0
2.您對餐點(量與質)的滿意程度	62.7	25.4	11.9	0	0
工作					
1.本次研習班內容與 您目前工作相關的程度	78.0	22.0	0	0	0
2.本次研習班對 您目前工作的幫助程度	79.7	18.6	1.7	0	0

(7月11日星期六)

	非常滿意 10 9	滿意 8 7	普通 6 5	不滿意 4 3	差 2 1
講師一：HAART藥物副作用之評估與處理 (洪健清 醫師)					
1.能清楚表達及解說議題	84.7	13.6	1.7	0	0
2.能適當反應及回答問題	86.4	11.9	1.7	0	0
3.能客觀而不帶偏見的講解	84.7	13.6	1.7	0	0
4.教材及幻燈片內容	86.4	10.1	1.7	0	0
5.授課時間控制	79.7	20.3	0	0	0
講師二：女性(懷孕)感染者諮商會談技巧 (賴怡因 個管師)					
1.能清楚表達及解說議題	62.7	33.9	3.4	0	0
2.能適當反應及回答問題	59.3	37.3	3.4	0	0
3.能客觀而不帶偏見的講解	52.5	44.1	3.4	0	0
4.教材及幻燈片內容	61.0	33.9	5.1	0	0
5.授課時間控制	64.4	30.5	5.1	0	0

	非常滿意 10 9	滿意 8 7	普通 6 5	不滿意 4 3	差 2 1
講師三：懷孕婦女愛滋寶寶照護 (施鐘卿 個管師)					
1.能清楚表達及解說議題	78.0	20.3	1.7	0	0
2.能適當反應及回答問題	79.7	18.6	1.7	0	0
3.能客觀而不帶偏見的講解	79.7	18.6	1.7	0	0
4.教材及幻燈片內容	78.0	20.3	1.7	0	0
5.授課時間控制	78.0	20.3	1.7	0	0
講師四：個案管理實務綜論 （柯乃熒 教授）					
1.能清楚表達及解說議題	71.2	28.8	0	0	0
2.能適當反應及回答問題	71.2	28.8	0	0	0
3.能客觀而不帶偏見的講解	72.9	27.1	0	0	0
4.教材及幻燈片內容	72.9	27.1	0	0	0
5.授課時間控制	72.9	27.1	0	0	0
講師五：個案管理實務分享 (柯乃熒 教授)					
1. 與您目前工作相關的程度	74.6	23.7	1.7	0	0
2.對您目前工作的幫助程度	74.6	23.7	1.7	0	0
3.題目選擇	76.3	22.0	1.7	0	0
4.討論內容	74.6	23.7	1.7	0	0
5.時間控制	74.6	23.7	1.7	0	0
（7月12日星期日）					
講師六：性病評估與處置 (王建淳 醫師)					
1.能清楚表達及解說議題	84.7	15.3	0	0	0
2.能適當反應及回答問題	84.7	15.3	0	0	0
3.能客觀而不帶偏見的講解	83.1	16.9	0	0	0
4.教材及幻燈片內容	84.7	15.3	0	0	0
5.授課時間控制	81.4	18.6	0	0	0
講師七：(即將)旅外感染者照護 (李欣純 醫師)					
1.能清楚表達及解說議題	61.0	35.6	3.4	0	0
2.能適當反應及回答問題	62.7	33.9	3.4	0	0
3.能客觀而不帶偏見的講解	66.1	28.8	5.1	0	0
4.教材及幻燈片內容	64.4	28.8	3.4	3.4	0
5.授課時間控制	64.4	28.8	5.1	1.7	0

	非常滿意 10 9	滿意 8 7	普通 6 5	不滿意 4 3	差 2 1
講師八：愛滋修法之重點 (林宜慧 秘書長)					
1.能清楚表達及解說議題	57.6	35.6	6.8	0	0
2.能適當反應及回答問題	57.6	37.3	5.1	0	0
3.能客觀而不帶偏見的講解	57.6	37.3	5.1	0	0
4.教材及幻燈片內容	57.6	37.3	5.1	0	0
5.授課時間控制	57.6	37.3	5.1	0	0
講師九：感染者安置與資源轉介 (徐森杰 秘書長)					
1.能清楚表達及解說議題	79.7	20.3	0	0	0
2.能適當反應及回答問題	78.0	22.0	0	0	0
3.能客觀而不帶偏見的講解	78.0	22.0	0	0	0
4.教材及幻燈片內容	76.3	23.7	0	0	0
5.授課時間控制	78.0	22.0	0	0	0
總評					
1.您在本研習班整體收獲的滿意程度	81.4	18.6	0	0	0

2.本次研習班需改進的地方:

教材講義部份:

部分講師簡報與講義不一致。 若講師內容真的很多也很重要，可多安排上課時間，不要每個講師相同時間，但內容部份塞很多，感覺上課吸收效果不佳(以講師八為例)。OK、都很好，辛苦了!有少。更新不及。

內容結構部份:OK很完整。

講師教學部份: OK 時間控制。

其他建議事項:

茶水太淡，實在可以再友善點。小組活動的人數建議少點人。第一天太緊湊。OSCE各項重點提醒。

3.下一次研習班您最希望聽到的議題為:

困難個案的諮商技巧(X2)。個案管理師的心理衛生。娛樂性用藥與HAART(x5)。旅遊醫學相關議題。青少年娛樂性用藥文化。(旅外)教導病人上網買藥、旅行注意事項。藥物。愛滋法律問題。感染者國外就醫/藥物取得。皆可。會談技巧(X2)。伴侶婚姻諮商。照護經驗案例分享，採Q&A方式。直接與講師互動，能採小組進行，效果可能更好。(希望是多專業)。想多聽異性戀患者在患病後的情形和MSM的不同在哪?照顧HIV家人的經驗,如何支持?要點)。毒品。藥癮(娛樂性用藥)。

4.下一次研習班您最希望聽到那一位講師的演講:

蔡茂松(X2)。洪健清(X3)、王建淳、李欣純、巫沛瑩、謝佳吟、李夢萍(X2)、希望具有醫療法背景之律師。皆可。施鐘卿。徐森杰。

中華民國104年7月12日

附件一~3

感染症專科醫師愛滋病治療專業能力教育訓練研習班

104年度課程評量統計表

	非常滿意 10 9	滿意 8 7	普通 6 5	不滿意 4 3	差 2 1
課程					
1.時間安排控制	66.0%	27.6%	6.4%	0%	0%
2.講義教材	61.7%	34.0%	4.3%	0%	0%
3.研習班內容的結構性	68.1%	29.8%	2.1%	0%	0%
場地餐宿					
1.場地設施、空間的滿意程度	53.2%	40.4%	6.4%	0%	0 %
2.您對餐點(量與質)的滿意程度	53.2%	42.5%	4.3%	0%	0%
工作					
1.本次研習班內容與 您目前工作相關的程度	76.6%	23.4%	0%	0%	0%
2.本次研習班對 您目前工作的幫助程度	78.7%	21.3%	0%	0%	0%

(9月12日星期六)

	非常滿意 10 9	滿意 8 7	普通 6 5	不滿意 4 3	差 2 1
講師一：Update on HIV virology: diagnosis and monitoring (張淑媛 教授)					
1.能清楚表達及解說議題	70.2%	29.8%	0%	0%	0%
2.能適當反應及回答問題	66.0%	34.0%	0%	0%	0%
3.能客觀而不帶偏見的講解	70.2%	29.8%	0%	0%	0%
4.教材及幻燈片內容	63.8%	36.2%	0%	0%	0%
5.授課時間控制	66.0%	34.0%	0%	0%	0%
講師二：Introduction to combination antiretroviral therapy (吳冠陞 醫師)					
1.能清楚表達及解說議題	83.0%	17.0%	0%	0%	0%
2.能適當反應及回答問題	85.1%	14.90%	0%	0%	0%
3.能客觀而不帶偏見的講解	85.1%	14.90%	0%	0%	0%
4.教材及幻燈片內容	85.1%	14.90%	0%	0%	0%
5.授課時間控制	83.0%	17.0%	0%	0 %	0%

	非常滿意 10 9	滿意 8 7	普通 6 5	不滿意 4 3	差 2 1
講師三：Management of opportunistic infections and malignancy: gastrointestinal and others (1) (蔡茂松 醫師)					
1.能清楚表達及解說議題	40.4%	46.8%	10.6%	2.1%	0%
2.能適當反應及回答問題	40.4%	51.1%	6.4%	2.1%	0%
3.能客觀而不帶偏見的講解	42.6%	51.1%	6.4%	0%	0%
4.教材及幻燈片內容	40.4%	38.3%	6.4%	0%	0%
5.授課時間控制	46.8%	46.8%	6.4%	0%	0%
講師四：Management and prophylaxis of opportunistic infections and malignancy: respiratory and nervous systems (1) (鄭健禹 醫師)					
1.能清楚表達及解說議題	31.9%	51.1%	12.8%	2.1%	2.1%
2.能適當反應及回答問題	34.0%	51.1%	10.6%	2.1%	2.1%
3.能客觀而不帶偏見的講解	44.7%	42.6%	10.6%	0%	2.1%
4.教材及幻燈片內容	31.9%	46.8%	19.1%	0%	2.1%
5.授課時間控制	40.4%	46.8%	8.5%	2.1%	2.1%
講師五：Hepatitis co-infections: epidemiology, prevention and management (顧文瑋 醫師)					
1.能清楚表達及解說議題	76.6%	23.4%	0%	0%	0%
2.能適當反應及回答問題	72.3%	27.7%	0%	0%	0%
3.能客觀而不帶偏見的講解	74.5%	25.5%	0%	0%	0%
4.教材及幻燈片內容	74.5%	25.5%	0%	0%	0%
5.授課時間控制	74.5%	25.5%	0%	0%	0%
講師六：Complications of combination antiretroviral therapy (黃俊凱 醫師)					
1.能清楚表達及解說議題	78.7%	19.1%	2.1%	0%	0%
2.能適當反應及回答問題	78.7%	19.1%	2.1%	0%	0%
3.能客觀而不帶偏見的講解	76.6%	23.4%	0%	0%	0%
4.教材及幻燈片內容	78.7%	21.3%	0%	0%	0%
5.授課時間控制	76.6%	23.4%	0%	0%	0%
講師七：Transmitted drug resistance: update and management (蔡宏津 醫師)					
1.能清楚表達及解說議題	63.8%	31.9%	4.3%	0%	0%
2.能適當反應及回答問題	68.1%	27.7%	4.3%	0%	0%
3.能客觀而不帶偏見的講解	66.0%	31.9%	2.1%	0%	0%
4.教材及幻燈片內容	61.7%	34.0%	4.3%	0%	0%
5.授課時間控制	68.1%	27.7%	4.3%	0%	0%

	非常滿意 10 9	滿意 8 7	普通 6 5	不滿意 4 3	差 2 1
講師八：Case demonstration(1)–respiratory and nervous system (盛望徽 醫師)					
1.能清楚表達及解說議題	74.5%	23.4%	2.1%	0%	0%
2.能適當反應及回答問題	72.3%	25.5%	2.1%	0%	0%
3.能客觀而不帶偏見的講解	72.3%	27.7%	0%	0%	0%
4.教材及幻燈片內容	70.2%	27.7%	2.1%	0%	0%
5.授課時間控制	70.2%	27.7%	2.1%	0%	0%
講師九：Case demonstration (2)-gastrointestinal and others (李佳雯 醫師)					
1.能清楚表達及解說議題	70.2%	27.7%	2.1%	0%	0%
2.能適當反應及回答問題	72.3%	25.2%	2.1%	0%	0%
3.能客觀而不帶偏見的講解	66.0%	34.0%	0%	0%	0%
4.教材及幻燈片內容	68.1%	31.9%	0%	0%	0%
5.授課時間控制	66.0%	34.0%	0%	0%	0%
(9月13日星期日)					
講師十：Management of pediatric patients with HIV infection: from birth to teenage (呂俊毅 醫師)					
1.能清楚表達及解說議題	74.5%	23.4%	2.1%	0%	0%
2.能適當反應及回答問題	74.5%	23.4%	2.1%	0%	0%
3.能客觀而不帶偏見的講解	70.2%	29.8%	0%	0%	0%
4.教材及幻燈片內容	72.3%	25.2%	2.1%	0%	0%
5.授課時間控制	61.7%	36.2%	2.1%	0%	0%
講師十一：Management of serodiscordant couple preparing for pregnancy and HIV- infected women with pregnancy (鄭舒倖 醫師)					
1.能清楚表達及解說議題	70.2%	29.8%	0%	0%	0%
2.能適當反應及回答問題	74.5%	25.5%	0%	0%	0%
3.能客觀而不帶偏見的講解	74.5%	25.5%	0%	0%	0%
4.教材及幻燈片內容	70.2%	27.7%	2.1%	0%	0%
5.授課時間控制	78.7%	21.3%	0%	0%	0%
講師十二：Management of common sexually transmitted infections (王建淳 醫師)					
1.能清楚表達及解說議題	83.0%	17.0%	0%	0%	0%
2.能適當反應及回答問題	83.0%	17.0%	0%	0%	0%
3.能客觀而不帶偏見的講解	85.1%	14.9%	0%	0%	0%
4.教材及幻燈片內容	85.1%	14.9%	0%	0%	0%
5.授課時間控制	83.0%	17.0%	0%	0%	0%

	非常滿意	滿意	普通	不滿意	差
	10 9	8 7	6 5	4 3	2 1

講師十三：Update of management of Pre- and post-exposure prophylaxis in occupation and non-occupational settings (楊家瑞 醫師)

1.能清楚表達及解說議題	80.9%	19.1%	0%	0%	0%
2.能適當反應及回答問題	76.6%	23.4%	0%	0%	0%
3.能客觀而不帶偏見的講解	76.6%	23.4%	0%	0%	0%
4.教材及幻燈片內容	74.5%	23.4%	2.1%	0%	0%
5.授課時間控制	76.6%	21.3%	2.1%	0%	0%

總評

1.您在本研習班整體收獲的滿意程度	89.4%	10.6%	0%	0%	0%

2.本次研習班需改進的地方:

教材講義部份: good,ok(x4),建議第九堂課希望能提供"講憶"。 須向講師確認紙本講義正確性/版本。power point是否可上傳網站。優。很舒服的大小，恰恰好。希望slide與紙本盡量符合。Clinical case presentation 的時間可以安排20-30mm即可，50mm太長了。

內容結構部份: good,ok(x4),蒂一日的蔡茂松醫師及鄭健禹醫師和盛醫師、李佳雯醫師部分有一些重覆，也許可合併。OI部分重覆性太高。優。涵蓋大部份的重點。

講師教學部份: good(x2),ok(x4),優。受益良多，很好的重點複習。建議鄭健禹醫師可不可以不要照本宣科念slide，感覺上對自己的slide內容 很不熟。而且缺乏整理。蔡宏津醫師講解得很仔細，但太多lab detail 了。建憶勝醫師可以多舉一些clinical 例子。

其他建議事項:課程內容非常豐富，但禮拜六上得有點宛，內容課程有些重複，可能可以再精簡一些，thank!建議第8湯客希望能以臨床案例為主而非知識性授課，第4、7堂課是否PPT字體可以放大一些。連續2年上課有相當多的knowledge更新，而講師們卻能Update資料，受益良多。可否提早check in。非常感謝學會和贊助者的用心。

3.下一次研習班您最希望聽到的議題為: 1.HIV & metabolic syndrome ;2.HIV infertility

4.下一次研習班您最希望聽到那一位講師的演講: 都希望聽到。Dr.洪健清(x4)、Dr.吳冠陞(x6)、Dr蔡茂松、Dr黃俊凱(x3)。林育蕙醫師(X2)。每位講師都很棒，收穫很多。 呂俊毅醫師(x3)。張淑媛老師(x4)。王建淳醫師(x3)。盛望徽醫師。鄭舒倖醫師 。

中華民國104年9月13日

附件一~4

台大醫院內科部「愛滋病防治中心」104 年度 HIV/AIDS 專題研討會

次數	日期	演講者	題目
1	1 月 6 日	蔡人植	HIV-HCV co-infection in high risk population
2	1 月 20 日	孫幸筠	Tronsplantion in HIV-infected patients
3	1 月 27 日	施鐘卿	HIV 孕婦生產之個案管理處理案例分析
4	2 月 17 日	張莉容	協助個案轉介做生殖醫學之案例分享
5	2 月 17 日	張君俞	協助個案轉介做生殖醫學之案例分享
6	3 月 24 日	林綺英	Herpes zoster among the HIV-infected patiens who recently initiated HAART at NTUH 2012-2014
7	3 月 31 日	廖敏伶	愛滋實務經驗-娛樂性用藥(安非他命)
8	4 月 14 日	鄒華春	HPV 在青少年同志人群中的感染
9	4 月 21 日	葉淨元	Thyroid Disease in patients infected with HIV
10	4 月 28 日	楊上平	第四類處方申請經驗分享
11	5 月 5 日	羅一鈞	HIV 感染者合併急性 C 肝病毒感染的流行病學
12	5 月 19 日	羅玉珍	愛滋病毒感染者憂鬱量表分析
13	5 月 26 日	賴宗志	匿名篩檢抗藥性研究
14	6 月 2 日	張淑媛	目前抗藥性研究成果
15	6 月 9 日	巫沛瑩	困難 HIV 個案處置經驗
16	6 月 23 日	徐志雲	HIV 感染者常見之精神疾患
17	6 月 30 日	楊家瑞	Current dilemna in the maragement ofsyholis
18	7 月 7 日	劉尊榮	A 54yr hetrosexual male with prersistent generalized LAP and acute neutropenia
19	9 月 15 日	巫沛瑩	Indicators of quality of HIV care of NTUH:now & the future
20	9 月 22 日	孫幸筠	器官捐贈意願於 HIV 感染病患
21	10 月 6 日	楊睿濬	HIV Journal reading--HCV/JIV
22	10/14 日	林冠吟	Trends and outcomes of late ART initation among HIV-positive Taiwanese patients
23	10/27 日	呂孟凡	Nutrition therapy of Metabolic syndrome
24	11/3 日	陳冠宇	HAV among our HIV-positive patients
25	11/10 日	黃怡嘉	B 肝疫苗接種的效率
26	11/17 日	蔡茂松	2012 年後台大醫院用藥分析

地點：台大醫院西址綜合病房討論室(舊五東病房三樓，由萊爾富便利商店上樓)

附件二

計畫編號：MOHW104-CDC-C-114-000301

衛生福利部疾病管制署 104 年委託科技研究計畫

計畫名稱：愛滋病毒感染之體液暴露後預防性投藥效果之長期前瞻性研究

年 度 研 究 報 告

執行機構：台大醫院愛滋病防治中心

計畫主持人：楊家瑞

研究人員：李幸娟

執行期間：104 年 01 月 01 日至 104 年 12 月 31 日

＊本研究報告僅供參考，不代表本署意見，如對媒體發布研究成果應事先徵求本署同意*

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壹、中文摘要

後天免疫缺乏症候群(愛滋病,Acquired Immunodeficiency Syndrome, AIDS),自從1981年在美國發現以來,已成為全世界二十一世紀最重要的公共衛生問題,國內自 1984 年首例迄今,已逾二萬三千名以上被診斷和通報,由於感染人數持續增加,而且年齡層逐漸下降,早已經是衛生署傳染病防治工作的重要課題之一。愛滋病是由人類免疫缺乏病毒(Human Immunodeficiency Virus, HIV)透過血液或體液接觸而所傳染,全球各地主要之流行途徑大多是經由性行為,因此亦為性病之一,防治之法無他,即倡導安全性行為之重要性,以及教導高危險群定期檢驗追蹤;已被感染者若能及早發現,一方面需要追蹤治療,另一方面藉由 100% 之安全性行為,防堵已感染者將愛滋病毒進一步傳播。因此呼籲經常無保護措施性行為者、且性伴侶眾多者接受篩檢與專業心理諮詢,是非常重要的。根據衛生署的統計資料顯示,平均每 2~3 個小時發現 1 名新感染者,因此提供一個可信賴的 HIV 體液暴露者篩檢與專業心理諮詢的管道,應是杜絕愛滋病傳播最重要而且有效的方法,並可讓感染者有及早接受治療的機會。此外,目前已有多項國外觀察性研究顯示,體液暴露後預防性投藥可減低愛滋病毒感染的風險,因此,我們還可針對特定高危險族群(如男同志)在發生性行為發生體液暴露後,除了提供諮商以外,我們還可以提供相關性病的檢驗和治療,並且進一步評估是否有需要建議使用暴露後預防性投藥以降低愛滋病毒傳染的風險。

本計畫將針對特定高風險族群等非職業性高危險愛滋病毒體液暴露者建立完整且統一的愛滋病毒體液暴露事件處理流程。提供愛滋病毒篩檢及專線諮詢與衛教服務,以降低愛滋病毒感染的機會及減輕諮詢者其不安及焦慮。此外,於適當評估體液暴露感染之風險並提供愛滋病毒之快速篩檢,確定為非愛滋病毒感染者後,於 48 至 72 小時內給予及時之自費預防藥物,以發揮有效之防疫功能。

在本研究中,自 2011 年 3 月起至 2014 年 10 月止共有 296 人次接受暴露後預防性投藥,全部的人均在 72 小時之內投藥(其中 88.6%的人在 48 小時內即接受投藥),完成 28 天投藥的比例為 84.8%,對於處方之藥物在 2013 年以 TDF+3TC+LPV/r 為主,其次則為 TDF+3TC+RAL,大致上耐受性良好。追蹤至

2014 年 10 月底，僅有一人為血清陽轉成 HIV 感染者，但經評估顯示其陽轉與病人本身之高風險性行為模式有關，而非藥物預防失敗。

關鍵詞：愛滋病毒感染、暴露後預防性投藥、愛滋病毒檢驗、抗愛滋病毒藥物

貳、本文

一、前言

後天免疫缺乏症候群(愛滋病,Acquired Immunodeficiency Syndrome, AIDS),自從1981年在美國發現以來,已成為全世界二十一世紀最重要的公共衛生問題,國內自 1984 年首例迄今,已逾二萬三千名以上被診斷和通報,由於感染人數持續增加,而且年齡層逐漸下降,早已經是衛生署傳染病防治工作的重要課題之一。愛滋病是由人類免疫缺乏病毒(Human Immunodeficiency Virus, HIV)透過血液或體液接觸而所傳染,全球各地主要之流行途徑大多是經由性行為,因此亦為性病之一,防治之法無他,即倡導安全性行為之重要性,以及教導高危險群定期檢驗追蹤;已被感染者若能及早發現,一方面需要追蹤治療,另一方面藉由 100% 之安全性行為,防堵已感染者將愛滋病毒進一步傳播。因此呼籲經常無保護措施性行為者、且性伴侶眾多者接受篩檢與專業心理諮詢,是非常重要的。根據衛生署的統計資料顯示,平均每 2~3 個小時發現 1 名新感染者,因此提供一個可信賴的 HIV 體液暴露者篩檢與專業心理諮詢的管道,應是杜絕愛滋病傳播最重要而且有效的方法,並可讓感染者有及早接受治療的機會。此外,目前已有多項國外觀察性研究顯示,體液暴露後預防性投藥可減低愛滋病毒感染的風險,因此,我們還可針對特定高危險族群(如男同志)在發生性行為發生體液暴露後,除了提供諮商以外,我們還可以提供相關性病的檢驗和治療,並且進一步評估是否有需要建議使用暴露後預防性投藥以降低愛滋病毒傳染的風險。

本計畫將針對特定高風險族群等非職業性高危險愛滋病毒體液暴露者建立完整且統一的愛滋病毒體液暴露事件處理流程。提供欲至亞東醫院尋求協助的個案專線諮詢與衛教服務,並適當評估體液暴露感染之風險並提供愛滋病毒之快速篩檢,確定為非愛滋病毒感染者後,於48至72小時內給予及時之自費預防藥物,以發揮有效之防疫功能。

二、材料與方法

執行期間: 2015 年 1 月 1 日迄 2015 年 12 月 31 日。

研究方法:

實施方式:

(一) 利用國內外資訊管道蒐集各種相關文獻，並彙集其他國家對於疑似愛滋病毒體液暴露後事件處理流程等資料。成立「愛滋病毒體液暴露諮詢及篩檢」專線。提供問題解決的管道，透過諮詢過程加強對傳染病防治的認識，積極採取預防措施，降低感染率。安排相關人員接受訓練及在職教育，加強電話中處理方法及程序的一致性。

(二) 加強高風險族群愛滋病毒防治之宣傳。印製愛滋病毒感染服藥相關衛教之資料提供暴露個案參考。內容包括服務時間、愛滋病疑問、如何預防及檢驗等。提供快速且正確的檢驗、診斷及治療，降低感染的機會。

(三) 提供篩檢前之諮詢服務：將進行工作人員訓練，使其具有諮詢服務之能力。諮詢服務之內容：清楚解釋「愛滋病」及「愛滋病毒檢驗」、空窗期與潛伏期的意義、愛滋病的主要傳染途徑、愛滋病的預防方法、「全程」使用保險套的「安全性行為」及「比較安全性行為」觀念、愛滋病病毒檢驗的功能、限制以及如何獲知檢驗結果。電話先由護理師予以回答，依其嚴重性決定是否轉介給醫師。若有必要時可立即諮詢醫師在門診時間內予診斷及治療。

(四) 檢驗方法：以酵素免疫反應法及顆粒凝集法(Particle Agglutination, PA)進行初步篩檢，呈陽性反應者，再採檢體並重複酵素免疫反應法與西方墨點法，皆為陽性者為確認個案。。檢驗結果由醫師給予必要的檢驗後諮商。陽性反應者請回院門診，並填報「傳染病個案報告單」。

(五) 針對 HIV 體液暴露後之高風險族群，提供自費之藥物預防，並追蹤血清之變化諮詢記錄及處理追蹤結果可作為相關單位及學術機構研究發展之用，作為爾後政策擬定之參考。追蹤之期程為暴露後 6 周，3 個月及 6 個月。同時於第一次檢驗時，檢查其他性病如梅毒，並送驗尿液檢體至疾病管制局檢驗淋病及披衣菌尿道炎。進行流程如附表二

(六) 教育及資料更新：提供對 HIV 指定照顧醫院對 HIV 體液暴露之諮詢教育，且參考國內外文獻及指引更新 HIV 體液暴露預防流程及藥物，定期舉辦研討會提供 HIV 體液暴露處理相關資訊。

表二 暴露後血液檢查及時程

	基礎值	暴露後二周	暴露後六周	暴露後三個月	暴露後六個月
HIV1/2 antibody (EIA)$	V		V	V	V
Complete blood count with differential	V	V			
Serum liver enzymes: AST ALT	V	V			
Urine PCR for chlamydia and gonorrhea	V				
Serologic tests for syphilis: VDRL/RPR, TPHA	V	V	V		
HBsAg, anti-HBs#	V		V	V	
HCV antibody test (e.g., EIA/ELISA)*	V			V	V
Pregnancy test (urine and blood)	V	V	V		

三、結果

由於本次前瞻性研究之 IRB 於 2015 年 05 月 05 日通過可正式收案，因此自 2015 年 05 月 05 日至 2015 年 10 月 31 日止，共有 50 人次至板橋亞東醫院接受暴露後預防性投藥，全部的人均在 72 小時之內投藥，完成 28 天投藥的比例為 100%。對於處方之藥物主要是以 TDF+3TC (或 TDF/FTC) +RAL 為主，其次則為 TDF+3TC (或 TDF/FTC) +LPV/r，大致上以 RAL-based regimen 副作用較小。追蹤至 2015 年 10 月底，並無血清陽轉成 HIV 感染者。

由附表一顯示，尋求預防性投藥者大部份為男性，但男同性戀者約佔所有人的 72.0%，有 74.0%的人是透過網路尋求一夜情對象，另外則有 22.0%是找性工作者，92.0%的人學歷在大學以上，有 82.0%的人在過去三個月內至少有過兩個性伴侶，而這些高危險族群中僅 48%曾經做過 HIV 篩檢，此外，有 64.0%在過

去三個月內戴保險套的比例低於 50%。由於 nPEP 已推行數年，因此共有約 18% 的個案過去曾經接受過預防性投藥。

四、討論

從目前初步的結果可以看出，暴露後預防性投藥在高風險族群中，具有一定的成效，就如同國外的觀察性研究一般。到目前為止， 無個案真正 HIV 陽轉。一部份的原因可能是藥物本身的預防成效，但另一方面則也有可能是因單次性行為其實感染率就文獻統計而言並不高，再加上來源個案的感染狀態絕大多數不明，因此沒有 HIV 感染。然而，我們已經觀察到，重複接受 nPEP 的比例高達 18%，顯示我們仍需仰賴針對這一些個案的衛教諮詢，改變他們錯誤的觀念，方能在預防上達到最好的成效，更進一步可結合目前國際上推行的暴露前預防性投藥。此外，如何針對網路一夜情進行宣導，減少高風險性行為的比例，將是未來在防疫上重要的一環。

五、結論

就初步的結果而言，暴露後預防性投藥有其效果，但絕非單一之預防方式，而應整合將個案連結進入衛教諮詢的系統，方可在 HIV 防疫上收到好的成效；針對難以抑制慾望而反覆發生高風險行為者，可考慮建議轉為暴露前預防性投藥。此外，防疫政策上仍須針對網路尋求一夜情的族群進行預防政策及宣導的擬定。

對政策之具體建議:防疫政策上有需要暴露後預防性投藥指引之實施，並針對網路尋求一夜情的族群進行暴露後預防性投藥政策的宣導。

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參、圖表

Table 1. Baseline characteristics of the cases who sought for nPEP of HIV

Characteristics	Numbers (%)
Sex	
Male	48 (96.0)
Female	2 (4.0)
Risk behavior	
MSM	36 (72.0)
non-MSM	14 (28.0)
Source cases	
Internet	37 (74.0)
Sex workers	11 (22.0)
Others	2 (4.0)
Education: college or higher	46 (92.0)
Marriage	
Yes	4 (8.0)
No	46 (92.0)
History of STDs	6 (12.0)
Illicit drug use	8 (16.0)
Sex partners in the past 3 months	
Partner ≤1	9 (18.0)
Partners: 2-5	37 (74.0)
Partners >5	4 (8.0)
History of nPEP	9 (18.0)
History of HIV screening	24 (48.0)
Condom use in the past 3 months: <50%	32 (64.0)
Completion of nPEP	50 (100)
nPEP regimen	
AZT-based	7 (14.0)
TDF-based	43 (86.0)

附件三

計畫編號：MOHW104-CDC-C-114-000301

衛生福利部疾病管制署 104 年委託科技研究計畫

計畫名稱：台灣地區 HIV 感染者抗愛滋病毒藥物服藥後的抗藥性長期前瞻性研究

年 度 研 究 報 告

執行機構：國立台灣大學醫學院醫學檢驗暨生物技術學系

計畫主持人：張淑媛

研究人員：洪健清、張淑芳、蘇意青

執行期間：104 年 01 月 01 日至 104 年 12 月 31 日

＊本研究報告僅供參考，不代表本署意見，如對媒體發布研究成果應事先徵求本署同意*

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壹、中文摘要

本計畫主要探討台灣地區未接受三合一雞尾酒療法病人，其病毒抗藥性的基因型盛行率。過去十個月，我們已經收納 553 位尚未接受三合一雞尾酒療法患者的檢體，進行原生性 HIV-1 病毒基因型抗藥性分析；其中 483 位愛滋病毒感染者的 HIV-1 病毒基因型抗藥性分析已完成。這些患者對於任一類藥物具有抗藥性的比例為 14.5%；其中針對蛋白酶抑制劑 (protease inhibitor)、核苷酸類似物的反轉錄酶抑制劑 (nucleoside-analogue reverse transcriptase inhibitors, NRTIs)、非核苷酸類似物的反轉錄酶抑制劑 (non-nucleoside-analogue reverse transcriptase inhibitors, NNRTIs)藥物的抗藥性基因型盛行率分別為 4.6%、2.5%、及 15.2%。對於兩種以上藥物具抗藥性的抗藥性基因型盛行率是 1.4%。在分析同時，我們也收到 245 位接受三合一雞尾酒療法失敗患者的檢體，我們進行一般性 HIV-1 病毒基因型抗藥性分析；其中 196 位愛滋病毒感染者的 HIV-1 病毒基因型抗藥性分析已完成。這些病人中有 107 位(54.6%)對於任一類藥物具有抗藥性基因突變；其中針對蛋白酶抑制劑、核苷酸類似物的反轉錄酶抑制劑、非核苷酸類似物的反轉錄酶抑制劑藥物的抗藥性基因型盛行率分別為 4.6%、37.2%、44.9%；而同時具有兩種以上抗病毒藥物的抗藥性基因突變的比例為 31.1%。

關鍵詞：人類免疫不全病毒(HIV-1)、病毒抗藥性分析、蛋白酶、反轉錄酶、嵌合酶

貳、英文摘要

This study was aimed to determine the prevalence of transmitted drug resistance among treatment-naive HIV-1 infected patients in Taiwan. In the past ten months, we have received 553 specimens for analysis and among those, we have completed analysis of 483 specimens. The prevalence of transmitted drug resistance to any class of drugs among treatment-naive HIV-1 infected patients is 14.5%. The prevalence of resistance to protease inhibitor class, nucleoside-analogue reverse transcriptase inhibitors (NRTIs) class, and non-nucleoside-analogue reverse transcriptase inhibitors, (nNRTIs) are 4.6%、2.5% and 15.2%. The prevalence of resistance to more than two classes of drugs is 1.4%. In the mean time, we have received 245 specimens from treatment-experienced patients and among those, we have completed analysis of 196 specimens. The prevalence of transmitted drug resistance to any class of drugs among treatment-experienced HIV-1 infected patients is 54.6%. The prevalence of resistance to protease inhibitor class, nucleoside-analogue reverse transcriptase inhibitors (NRTIs) class, and non-nucleoside-analogue reverse transcriptase inhibitors, (nNRTIs) are 4.6%、37.2% and 44.9% . The prevalence of resistance to more than two classes of drugs is 31.1%.

關鍵詞：HIV-1、drug resistance、protease inhibitor、reverse transcriptase、integrase

參、本文

（一）前言

藥物治療對於受人類免疫不全病毒感染的患者已有很大的成效，不僅可以延長病人的壽命，並可進一步幫助恢復部分受影響的免疫系統功能。目前，絕大多數的病毒抑制劑，是藉由抑制人類免疫不全病毒的 *pol* 基因上與病毒活性或複製相關的病毒酵素，來達到抑　制病毒生長的效果。依藥物抑制的病毒基因與機制可分為三大類。第一類主要是抑制病毒蛋白酶的活性(Protease inhibitor,PI)。第二類是以擬似核苷酸衍生物的方式，來抑制反轉錄腖的活性(nucleoside reverse transcriptase inhibitors,NRTIs)。第三類是以非擬似核苷酸衍生物的形式，來抑制反轉錄酶的活性(non-nucleoside reverse transcriptase inhibitors,NNRTIs)。近年來，由於三合一雞尾酒療法比使用單一病毒抑制劑更能有效地抑制病毒的感染，許多醫師開始使用兩種或者三種不同類別的病毒抑制劑來治療病人。但是，在服用藥物過程中，可能因為病毒快速產生變異及病人不依醫師指示定時服藥等因素，病毒會在患者體內衍生出抗藥性病毒株。這些抗藥性病毒株的產生，已知與病人體內的病毒量快速增加，有極高的相關性[1, 2]。它會使得患者體內的病毒無法被完全地抑制，進而嚴重地影響到治療的效果與治療所需的時間[3, 4]。更嚴重的是這些抗藥性病毒株的產生，會造成原生抗藥性病毒株的流行。根據最近歐美的研究指出，在北美及歐洲分別有百分之一至十一及百分之九至二十一的患者，是被原生抗藥性病毒株所感染[5-10]。而這些被原生抗藥性病毒株所感染的病人，其接受藥物療法的成效，比被一般無抗藥性病毒株所感染的病人為差。例如，被原生抗藥性病毒株所感染的病人，經藥物治療後，其體內病毒量降至 500 copies/ml 以下所需的時間平均為十二週。遠較被一般病毒株感染病人的五週為長[11]。因此，了解原生抗藥性病毒株的盛行率及其所抗藥的藥物種類，將可作為臨床醫師在做藥物選擇上的參考，並進一步節省醫療資源。因此，本調查將藉由分析蛋白酶、反轉錄酶、嵌合酶及副受器這些基因上與抗藥性相關的基因變異，來了解台灣地區原生抗藥性人類免疫不全病毒的盛行率。 希望研究成果未來能幫助節省醫療成本，並提高個案的有效治療。至於不同亞型或重組病毒株內，與抗藥性相關的基因變異，是否將異於目前根據 B 亞型所知的基因變異，也將一併被列入分析[12-14]。

（二）材料與方法

執行期間： 2015 年 1 月 1 日迄 2015 年 12 月 31 日。

研究方法

1. 受試者：

在 2015 年 1 月到 2015 年 12 月期間，年滿 20 歲的愛滋病毒感染者只要未曾接受過三合一雞尾酒療法。我們會分析檢體中病毒抗藥性基因型，並分析盛行率的趨勢以提供臨床醫師將來選擇治療藥物的參考；並藉由電腦程式 PHYLIP 將被用來作基因系統樹分析(phylogenetic analysis)，以決定抗藥性病毒株之間的相關性。。針對收集個案病患之基本資料，如性別、年齡、HIV 感染危險因子、HIV 感染時間、伺機性感染或腫瘤、接受治療之時間、CD4 淋巴球之變化、血清 HIV 病毒量之變化、合併伺機性感染之有無、是否死亡等等作資料整理登錄。最後我們會分析那些可能的危險因子，如性別、感染途徑、及病毒亞型等，與抗藥性基因型具有相關性。本研究業經台大醫院倫委會同意通過後執行，受試者必須填寫受試者同意書後方可以參加試驗。

2. 實驗室檢驗

我們自病人血漿中萃取病毒顆粒中的 RNA，經由反轉錄酶反應將 RNA 轉換為 cDNA，再以 PCR 反應來放大病毒的 *gag-RT* 可轉錄區域。這些 PCR 產物經由洋膠電泳純化後，將直接作核酸定序，以為進一步病毒基因序列的相關分析。有關抗藥性相關的基因變異，我們主要依據國際愛滋協會與美國之抗藥突變小組委員會 (international AIDS Society-USA mutations panel) 所訂定，與人類免疫不全病毒抗藥性相關的基因變異[15, 16] (http://www.iasusa.org/) 以及參考 the Stanford University HIV Drug Resistance Database(http://hivdb.stanford.edu)、Geno2pheno (http://www.genafor.org/index.php) 的抗藥性基因型分析。此外，一些新近鑑定的基因變異也會陸續地被列入研究分析中。分析結果我們會以一標準格式，以電子郵件寄給送件的臨床醫師，以為治療時藥物選擇或更換的參考。

3. 統計分析

我們會使用 SAS 統計軟體進行所有統計分析。

（三）結果

自今年一月一日起至今年十月三十一日止，自未接受過三合一雞尾酒療法的 HIV 感染者，我們一共收到 553 件血液檢體進行基因型抗藥性檢測，目前已完成 483 件檢體的 HIV-1 病毒基因型抗藥性分析。自三合一雞尾酒療法治療失敗的 HIV 感染者，我們一共收到 245 件血液檢體進行基因型抗藥性檢測，目前已完成 196 件檢體的 HIV-1 病毒基因型抗藥性分析。這些檢體來自全台各家醫院(圖一)，其中 91.9%(444 件)來自北部醫院，7.7%(37 件)來自南部醫院，0.4%(2 件)來自東部醫院。

在未接受抗反轉錄藥物治療的病人中，對任一類藥物具有抗藥性的病毒株其總體盛行率為 14.5%，對反轉錄酶抑制藥物(nucleoside reverse transcriptase inhibitors,NRTIs)、非擬似核苷酸衍生物的反轉錄酶抑制藥物(non-nucleoside reverse transcriptase inhibitors,NNRTIs) 及蛋白酶抑制劑 (protease inhibitor)的抗藥性病毒株的比例分別為 4.6%、2.5%、及 15.2%。對兩種以上藥物具抗藥性的比例為 1.4%。

在抗反轉錄藥物治療失敗病人檢體中，對任一類藥物具有抗藥性的病毒株其總體盛行率為 54.6%。對反轉錄酶抑制藥物(nucleoside reverse transcriptase inhibitors,NRTIs)、非擬似核苷酸衍生物的反轉錄酶抑制藥物(non-nucleoside reverse transcriptase inhibitors,NNRTIs) 及蛋白酶抑制劑 (protease inhibitor)的抗藥性病毒株的比例分別為 4.6%、37.2%、及 44.9%。對兩種以上藥物具抗藥性的比例為 31.1%。

由於在未接受抗反轉錄藥物治療的病人中，對任一類藥物具有抗藥性的病毒株其總體盛行率為 14.5%，相較於之前我們去年的調查，其抗藥性的比例有些微升高的趨勢(13.2% v.s. 14.5%,)(圖二)，主要升高的抗藥性藥物種類是蛋白酶抑制劑 (protease inhibitor) (2.5% v.s. 4.6%)及非擬似核苷酸衍生物的反轉錄酶抑制藥物(non-nucleoside reverse transcriptase inhibitors, NNRTIs) (9.4% v.s. 15.2%)產生抗藥性(圖二)。擬似核苷酸衍生物的反轉錄酶抑制藥物(nucleoside reverse transcriptase inhibitors, NNRTIs)及多重抗藥性病毒株的盛行率分別是些許下降(3.0% v.s. 2.5%)及 (1.6% v.s. 1.4%)。

在接受抗反轉錄藥物治療失敗的病人中，對任一類藥物具有抗藥性的病毒株其總體盛行率為 54.6%，相較於之前我們去年的調查，其抗藥性的比例有些微下降的趨勢(61.2% v.s. 54.6%,)(圖三)。蛋白酶抑制劑 (protease inhibitor) (7.1% v.s. 4.6%)、擬似核苷酸衍生物的反轉錄酶抑制藥物(nucleoside reverse transcriptase inhibitors, NNRTIs) (46.5% v.s.

37.2%,)、非擬似核苷酸衍生物的反轉錄酶抑制藥物(non-nucleoside reverse transcriptase inhibitors, NNRTIs) (45.9% v.s. 44.9%)及多重抗藥性病毒株的盛行率(34.1% v.s. 31.1%)都有下降的趨勢(圖三)。

（四）討論

今年的追蹤研究發現，在未接受抗反轉錄藥物治療的病人中，對任一類藥物具有抗藥性的病毒株其總體盛行率為 14.5%，相較於之前我們去年的調查，其抗藥性的比例有些許升高的趨勢(13.2 v.s. 14.5%)。進一步的研究發現，具原生性人類免疫不全病毒(HIV-1)抗藥性基因盛行率的上升主要是針對蛋白胨抑制劑(protease inhibitor)及非擬似核苷酸衍生物的反轉錄酶抑制藥物(non-nucleoside reverse transcriptase inhibitors,NNRTIs)這兩類藥物(圖二)。以 NNRTI 藥物為基礎之治療方案為目前世界上最常採用第一線 HIV-1 治療方案，同時為世界衛生組織(WHO)所建議之第一線雞尾酒療法藥物[31, 36-38]，同時也是台灣治療愛滋病人的第一線藥物。根據我們之前發表的論文評估，具傳播性之抗藥性病毒株在臨床現象所造成之影響，我們發現即使是對於一種抗反轉錄病毒藥物具有低程度抗性的傳播性抗藥性病毒株(基因型敏感性評分≦2.5；GSS≦2.5) ，比起不具有傳播性抗藥病毒株的治療效果在使用雞尾酒治療(cART)前兩年會導至大於五倍的治療失敗風險。此現象於接受以非擬似核苷酸衍生物的反轉錄抑制藥物(NNRTI)為主之雞尾酒療法的病患中特別顯著[17]。根據這些前人的研究及本計畫的研究結果，我們建議目前如果要接受 NNRTI 抗反轉錄病毒藥物治療前，應立即進行抗藥性檢測；或是建議使用蛋白胨抑制劑。因為在一大型研究第一線的雞尾酒療法針對具傳播力之抗藥性病毒株的影響之調查中，Wittkop 等人發現，以蛋白酶抑制藥物為基礎並帶有促進劑 Ritonavir 的治療方案相較於以 NNRTI 為基礎的治療方式在易感染或有潛力感染低程度抗藥性病毒的病患中擁有較佳病毒治療結果；因此，他們建議在抗藥性病毒株流行但無例行性基因檢測的區域應該考慮使用帶有促進劑的蛋白酶抑制藥物之第一線雞尾酒療法[18]。

（五）結論

對政策之具體建議:病人要服用以 NNRTI 為基礎的雞尾酒療法，應該在用藥前接受抗藥性基因檢測，以確保藥物治療的效果。

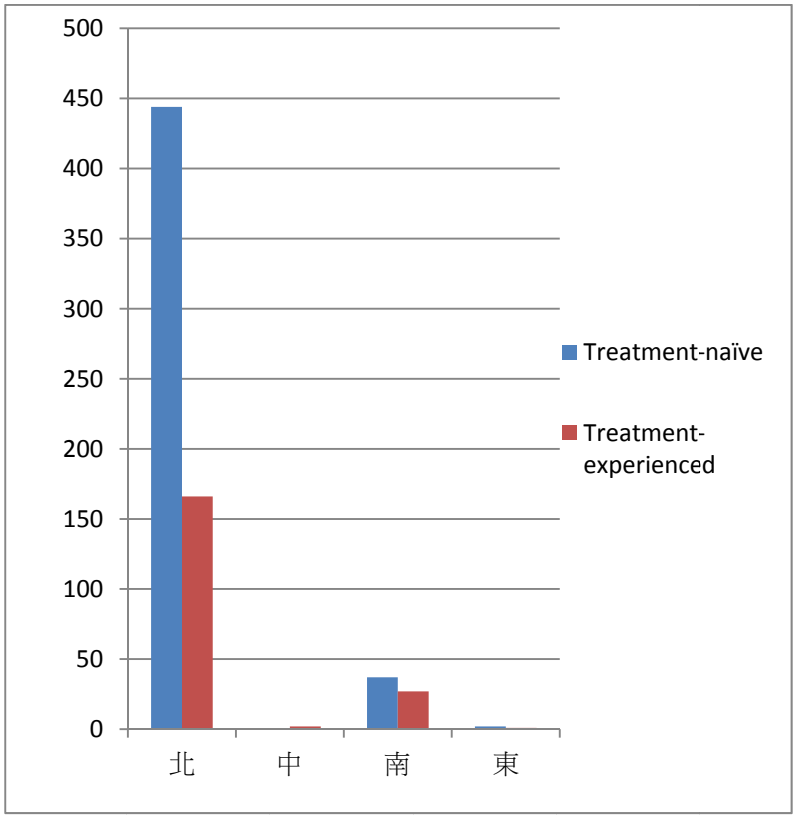
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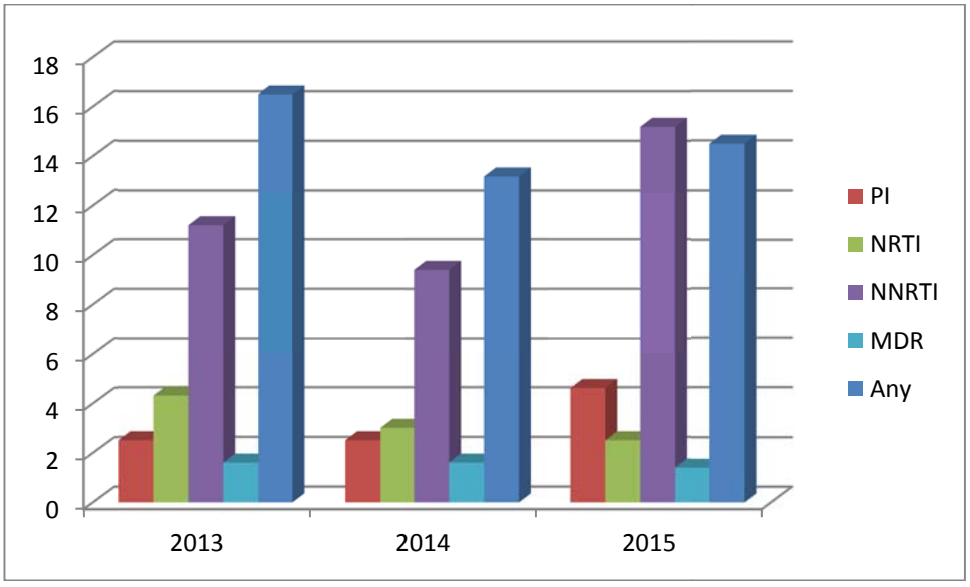
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（七）圖表

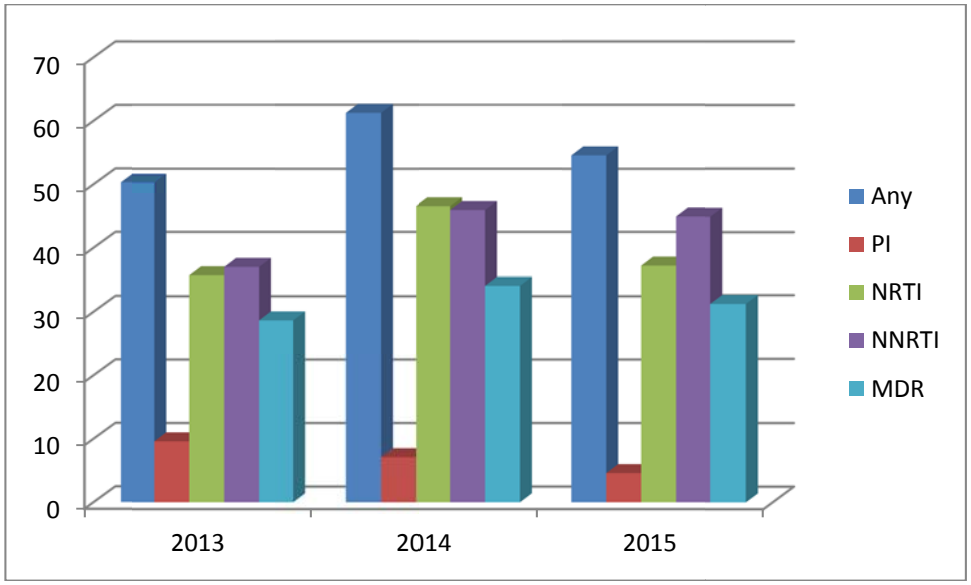
圖一、檢體來源分布圖



圖二、原生性抗藥性盛行率的趨勢圖



圖三、治療失敗患者抗藥性盛行率的趨勢圖



附件四

計畫編號：MOHW104-CDC-C-114-000301

衛生福利部疾病管制署 104 年委託科技研究計畫

計畫名稱：台灣地區 HIV 感染者藥品動態學和基因學研究

年 度 研 究 報 告

執行機構：台大醫院愛滋病防治中心

子計畫主持人：林淑文

研究人員：洪健清、張淑媛、蔡茂松、李官燁、唐新亞、劉玟君

執行期間： 104 年 1 月 1 日至 104 年 12 月 31 日

＊本研究報告僅供參考，不代表本署意見，如對媒體發布研究成果應事先徵求本署同意*

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壹、中文摘要

抗愛滋病毒藥物(ART)中,NNRTI 與 PI 等主要經由肝臟酵素 cytochrome P450(CYP 450）代謝。代謝酵素的活性與單一核苷酸基因多形性（single nucleotide polymorphism, SNP)、生理病理狀況、藥品交互作用息息相關，因此每個人代謝藥品的速率可能差異極大，可能影響治療效果、或增加劑量相關的毒性。因此監測藥物血中濃度極具臨床價值。治療 AIDS 相關之伺機性感染（opportunistic infection）的抗生素如 rifabutin 與 trimethoprim/sulfamethoxazole (cotrimoxazole)亦有這樣的特性。

本研究藉由前瞻性的觀察，以高效能液相層析儀（HPLC）追蹤血中濃度、基因多型性、療效與副作用等關係，以探索最適合國人的 ART 劑量、藥品交互作用時之劑量調整原則等課題，累積國內之本土經驗，增進病人用藥安全、達到最大的經濟效益。迄今已為 780 位服用 EFV、530 位服用 ATV 與 120 位服用行 NVP 的病人進行分析。

服用 EFV 者的藥物血中濃度差異性相當大,絕大部分的濃度高於治療指引建議的 1 μg/mL，因此針對使用 EFV 達 6 個月以上、血中濃度高於 2 μg/mL、病毒量低於 200 copies/mL 者，我們近來嘗試將其 EFV 劑量減半、密切追蹤血中濃度與臨床療效與副作用。由於 ATV 的血中濃度可能受 tenofovir (TDF)影響而降低，但本研究發現併用者的血中濃度並未較低，療效也良好。約 20%服用長效 NVP 病人在糞便中發現藥錠殘骸，但病毒量或免疫球上升等臨床療效並未較差。多數病人的 SMX-TMP 血中濃度達到文獻建議的治療範圍之內；SMX 代謝物與原型藥藥物血中濃度比值在不良反應的發生上有統計顯著差異；而電解質不平衡和 TMP 藥物濃度與使用劑量之間也有顯著關係。Rifabutin 只有一位病人檢測，血中濃度在正常範圍內。

在本研究室將繼續現行模式，除了提供全國各醫療院所常規監測 ART 與抗生素血中濃度的服務、嘗試開發以 HPLC 方法測定其他 ART，在進行臨床相關研究的同時，亦提供臨床醫師重要的藥品濃度訊息。

關鍵詞：抗愛滋病毒藥物、基因多型性、atazanavir、efavirenz, nevirapine, cotrimoxazole, rifabutin

英文摘要

Highly active antiretroviral therapy (HAART) uses combination therapy of multiple antiretroviral agents. It dramatically improves the clinical responses in patients infected with human immunodeficiency virus (HIV), and decreases the mortality and morbidity of acquired immunodeficiency syndrome (AIDS). Efavirenz and nevirapine (non-nucleoside reverse transcriptase inhibitors, NNRTI), lopinavir/ ritonavir (protease inhibitor, PI), and maraviroc (chemokine receptor type 5 antagonist, CCR5 antagonist) are metabolized by cytochrome P450 (CYP 450) enzymes. Atazanavir (PI) and raltegravir (integrase inhibitor) are metabolized via UDP-glucuronosyltransferase (UGT1A1) in the liver. The activities of abovementioned enzymes affect the metabolic rates of ART, and their activities are determined by the single nucleotide polymorphism (SNP), pathophysiological state, and drug interactions. Each individual may metabolize ART in different rates, which causes the variability in pharmacokinetic (PK) parameters. Serum drug concentration may decreases in extensive metabolizes, which leads to treatment failure or drug resistance. On the other hand, serum concentration may be elevated in slow metabolizers and result in better treatment outcomes and/or more dose-related toxicity. This phenomenon also applys to some antibiotics in the treatment of opportunistic infections in AIDS patients, such as rifabutin and trimethoprim/sulfamethoxazole (cotrimoxazole). Therefore, it is clinically valuable to measure the plasma concentration of these medications. Furthermore, generic ART have been available recently.

Measurement of plasma concentration would provide a security net to ensure effectiveness of safety of treatment. Previous studies used high performance liquid chromatography (HPLC) to quantify the plasma concentration of ART and other antibiotics in HIV patients. Unfortunately, the assay is not feasible to all hospitals in Taiwan. A "PK laboratory" for plasma concentration and SNP may be beneficial for our medical care. The purpose of this prospective clinical study is to evaluate the relationship between drug plasma concentration, SNP, and effectiveness and adverse event of ARTs. Results of this study will serve as guidance for dosing adjustment in clinical use of ARTs and for management of drug interactions in patients with HIV. We will share our observations and experiences at the National Taiwan University Hospital domestically and internationally through our publications in the future.

Our laboratory has established assay methods with HPLC for plasma concentrations of

efavirenz, nevirapine, atazanavir, co-trimoxazole and rifabutin in the past. We have monitored 780, 120, 530, 82, and 1 patient in this year prospectively. There is a significant inter-individual variation of plasma concentration of ARTs and antimicrobial agents for opportunistic infections. Therefore, measurement of plasma concentration should be emphasized in clinical practice in Taiwan. The continuous study in the next year may assure the availability of the PK lab, provide the data for clinicians as a reference for ART regimen adjustment, and crucial information for health authority for decision making.

貳、本文

（一）前言

自從台灣地區診斷第一例愛滋病毒感染迄今已超過二十年，抗愛滋病毒藥物（antiretroviral therapy, 簡稱 ART）問世後，愛滋病毒的感染受到良好的控制；由多種 ART 組合而成的高效能抗愛滋病毒療法（highly active antiretroviral therapy, 簡稱 HAART）更使治療效果大增，減少後天免疫不全症候群（AIDS）的發生與死亡率[1-3]。ART 中，非核苷酸反轉錄酶抑制劑（non-nucleoside reverse transcriptase inhibitor, NNRTI）、蛋白酶抑制劑（protease inhibitor, PI）、CCR5 抑制劑（chemokine receptor type 5 antagonist）等主要經由肝臟酵素 cytochrome P450（CYP 450）代謝；整合酶抑制劑（integrase inhibitor）主要經由肝臟尿苷二磷酸葡萄糖醛酸基轉移酶（UDP-glucuronosyltransferase, UGT1A1）進行葡萄糖醛酸反應（glucuronidation），上述酵素的活性影響 ART 的代謝速率，而酵素活性與單一核苷酸基因多形性（single nucleotide polymorphism, SNP）、生理病理狀況、藥品交互作用息息相關，因此每個人代謝藥品的速率可能差異極大，使藥物動力學（pharmacokinetics, PK）的特性相去甚遠。病人本身代謝快或酵素活性被其他藥物誘導（induction）時，藥物半衰期（half-life）減短、血中濃度（serum concentration）降低，可能影響治療效果，甚至促使愛滋病毒產生抗藥性；若代謝慢或酵素活性被抑制時，藥物之血中濃度及目標作用部位（targeted site of action）的濃度將提高，可能因此而增加療效、甚至增加劑量相關（exposure-related）的毒性。為確保療效，應密切追蹤 CD4 淋巴球數與血漿愛滋病毒量，甚至考慮進行藥物血中濃度監測及/或調整 ART 劑量，維持 ART 的最低血中濃度在特定數值之上以確保療效。美國國家衛生研究院的衛生署（Department of Health and Human Services, DHHS）愛滋病的治療指引中建議 ART 最低血中濃度應維持在特定數值之上以確保療效[]，例如：

- Efavirenz (EFV): ≥ 1000 ng/mL
- Nevirapine (NVP): ≥ 3000 ng/mL
- Amprenavir 或 fosamprenavir: ≥ 400 ng/mL
- Atazanavir (ATV): ≥ 150 ng/mL
- Indinavir: ≥ 100 ng/mL
- Lopinavir/ritonavir: ≥ 1000 ng/mL
- Nelfinavir: ≥ 800 ng/mL

- Saquinavir: ≥ 100-250 ng/mL
- Tipranavir: ≥ 20500 ng/mL

一般而言，ART 雖療效佳，但副作用卻難以避免，可能導致高膽紅素血症、糖尿病、血脂肪過高、骨質疏鬆等問題。加上病患壽命越漸增長，身體器官功能異常或老化相關的疾病也愈容易在感染者身上出現。因此，在病患照顧上，不僅應追蹤藥效與存活，監測生化值變化與觀察上述副作用以確保用藥安全亦是醫療照護的重點。特定 ART 的某些副作用與血中濃度有關，例如 EFV (商品名 Stocrit，希寧，NNRTI 類) 及 ATV（商品名 Reyataz，瑞塔滋，PI 類）即是。EFV 加上兩種核苷酸反轉錄酶抑制劑（abacvair + lamivudine；tenofovir + emtricitabine；tenofovor + lamivudine）是現今國內外愛滋病毒感染治療建議中的首選[4]，可以確保藥物長期使用的遵囑性（adherence）。但若血中濃度超過 4 μg/mL 的病患，發生中樞神經副作用的比例為血中濃度在 1-4 μg/mL 病患的 3 倍[5]。EFV 係由 CYP3A 和 CYP2B 酵素系統代謝，CYP2B (516 位點)的 SNP 會造成 EFV 在體內的代謝速度有差異[6,7]。ATV 約自 95 年正式在臺灣上市使用，與其他 PI 相較，ATV 較少發生代謝方面如高血脂症及脂肪分佈異常（lipodystrophy)等併發症；但臨床使用時，由於 ATV 和膽紅素同樣經過 UGT1A1 代謝，互相競爭的情況下，國內約有一半的患者在服用 ATV 一個月後，膽紅素會上升，5%左右的患者更會產生明顯的黃疸症狀。國外的案例報告發現 ATV 的血中濃度是影響高膽紅素血症的重要原因之一。由於目前已知 UGT1A1 有基因多形性（genetic polymorphism）的現象，酵素活性差（慢代謝，UGT1A1*28）者可能較傾向產生嚴重的高膽紅素血症。由於國人中約有 56%為正常代謝者，其餘為異質接合（heterozygous）或同質接合（homozygous），可能增加高膽紅素血症的發生率。因此，以往的研究強調監測血中濃度的重要性，避免 EFV 濃度高於 4 μg/mL 或 ATV 濃度高於 0.85 μg/mL[8-11]。

另一方面，ATV 雖然一天只需服用一次，但需要酸性環境以促進胃腸道的吸收，因此建議盡量與食物一起吃、或飯後立即服用，而且要避免與制酸劑或其他胃藥合併服用[4]。但國人服藥時常習慣與胃藥併服，因此 ATV 血中濃度是否有達到美國 DHHS 治療指引中建議的 0.15 μg/mL 以上，亟需確認。再者，ATV 主要經由肝臟酵素 CYP3A4 代謝，可能受許多藥品或食品的影響而改變代謝速率，國內臨床使用 ATV 時，似乎不必加上 ritonavir (RTV, boosted PI) 也能達到很好的療效，因此監測 ATV 血中濃度實屬必

要。某些西方研究建議適當的血中濃度範圍為 0.15 - 0.85 μg/mL，以免因藥物濃度過低、使愛滋病毒產生抗藥性；或因濃度過高、增加高膽紅素血症的產生。

如同 ATV，PI 類的 lopinavir/ritonavir 也都經由 CYP 3A4 代謝，RTV 可強效抑制 CYP3A4 與 2D6 的活性、卻能提高 UGT1A1 的活性。CYP3A4 的活性可能受許多藥品或食品的影響而改變代謝速率，國內臨床常使用 lopinavir/ritonavir，因此監測血中濃度實屬必要[4]。

治療愛滋病毒感染者的 TB 時，EFV 雖為首選藥物，但對妊娠第一期的孕婦、無法忍受 EFV 副作用者、或感染抗藥性的病毒時，NVP 應是合適的替代藥品。但 NVP 與 rifampin 併用時，NVP 血中濃度會下降 37~58%，且兩者都有皮膚過敏與肝毒性的副作用，雖然小型研究並未證實 NVP 與 rifampin 的交互作用會影響臨床療效，但南非的大型世代追蹤研究、泰國的隨機分配臨床試驗與印度的臨床試驗卻發現 nevirapine 與 rifampin 併用的治療效果不佳，比起 EFV 與 rifampin 併用之病毒學反應較差、死亡率也較高，血中濃度偏低可能是導致臨床反應不良的主因之一[12-15]。此外，長期併用 NVP 與 rifampin 的安全性有待研究，也不清楚是否會增加肝毒性。泰國比較 TB 患者開始服用 NVP 標準劑量（最初兩週每日 200 mg、接著每日 400 mg）或提高劑量（最初兩週每日 400 mg、接著每日 600 mg）後的血中濃度，發現 80%接受標準劑量的血中濃度過低，但接受較高劑量者易發生 NVP 過敏反應。因此建議亞洲人若已在服用 rifampin，由於 CYP3A4 與 CYP2B6 活性已被誘導，加入 NVP 之最初兩週不需要以低劑量開始，之後也不需要因藥動學特性而特別提高劑量，使用標準劑量即可[16-19]。

與 EFV 相似，NVP 除了經由 CYP3A4 代謝外，CYP2B6 也佔了重要的地位，而 CYP2B6 的單一核苷酸基因多形性使每個人代謝藥品的速率差異極大，日本人中約有 20%的人代謝較其他人慢[20]，而國人的比例不詳。若 CYP2B6 代謝能力差，藥物可能經由其他途徑例如 CYP3A4 代謝，而具有 SNP 的基因如 CYP3A4*1B、CYP3A5*3 及 MDR1 (multidrug resistance protein 1)也可能會造成代謝速度差異[21-22]，

新藥 raltegravir（RAL）為愛滋病毒的整合酶抑制劑（integrase inhibitor），主要經由肝臟 UGT1A1 進行葡萄糖醛酸反應（glucuronidation）而代謝，因此 UGT1A1 的誘導物（如 rifampin）會顯著降低 raltegravir 的血中濃度，使 raltegravir 的 AUC 下降 40％，trough 濃度下降 61％。若每次劑量由一般的 400 mg 提高為 800 mg、再併用 rifampin，RAL 的 AUC 雖比單獨使用 400 mg 上升 27％，但 trough 濃度仍下降 53％。因此，臨床上應盡

量避免此類的併用，以其他藥品取代使用；若一定要併用 RAL 與 rifampin 時，可提高 RAL 的劑量至 800 mg，並密切監測愛滋病毒量。其他 UGT1A1 的誘導物也會產生類似的效果，例如 rifabutin、EFV、含 ritonavir 的 tipranavir；若治療反應不如預期，需考慮交互作用可能導致的影響。如前所述，UGT1A1 有基因多形性，代謝慢（UGT1A1*28）的愛滋病毒感染者是否較可能因血中濃度過高而產生副作用有待密切追蹤[4,23]。

新藥 maraviroc（MVC）是 CCR5 抑制劑（chemokine receptor type 5 antagonist）中唯一上市的藥品，經由 CYP 3A4 及 CYP 3A5 代謝，同時亦為 P-glycoprotein 的受質；但本身不影響 CYP 3A4 及 P-glycoprotein（P-gp）的活性。併用其他的 CYP 3A4 抑制物（如 ritonavir 及其他 PI）或誘導物（EFV、etravirine、rifampin）時，MVC 的代謝速率會被影響，應盡量避免此種合併使用，但若有臨床治療需要時，必須視併用藥品的特性調整 MVC 的劑量，以維持血中濃度在 50 ng/mL 以上。仿單建議 MVC 與強效 CYP 3A4 誘導物（例如 rifampin）併用時，MVC 的 AUC 可能下降 64%，只要同時間未使用任何 CYP 3A4 抑制藥品，劑量就需加倍為每次 600 mg；若同時也併用強效的 CYP 3A4 抑制藥品，劑量則維持每次 300 mg。與抑制 CYP 3A4 效果較弱 rifabutin 併用時，只要沒有同時使用其他的 CYP 3A4 抑制物或誘導物，仍可用 MVC 的正常劑量每次 300 mg；若同時也併用強效的 CYP 3A4 抑制藥品，劑量則維持每次 150 mg；不論劑量如何改變，服藥頻率都維持為一天二次。由於 MVC 為新藥、且只適用於已產生抗藥性的愛滋病毒感染，文獻中缺乏國內病人依上述建議調整劑量後的治療效果與副作用，亟待以追蹤藥品交互作用、血中濃度、療效與副作用確認。此外，雖然 MVC 經由 CYP 3A4 代謝的比例是 CYP 3A5 的 25 倍，但由於 CYP 3A5 具基因多型性，且同質合子的 SNP 6986G (*3)在白人占 90%、黑人占 30%，而中國人占 73%；國內發現在 42 位健康受試者中有 39 人（93%）帶有 CYP 3A5*3 基因型，SNP 如何影響 MVC 的 PK 性質仍不得而知。而 P-gp 的生成是由 multidrug resistance-1 (MDR1)基因調控，但目前 MDR1 的 SNP 仍在發展中，初步研究顯示國人的 SNP 型態與比例和其他種族、甚至中國大陸或新加坡的華人均不太相同，因此 MVC 的 PK 與 SNP 相當值得探索[4,23]。

文獻中多使用高效能液相層析儀（high performance liquid chromatography, HPLC）檢測 ART 血中濃度[24-27]，但並非每個醫療院所都能進行此種檢驗方式，因此有必要成立一個『藥品濃度監測中心』（PK laboratory），協助各地的醫療人員監測 ART 血中濃度。並藉由追蹤血中濃度、基因多型性、療效與副作用等關係，探索最適合國人的 ART 劑

量、藥品交互作用時之劑量調整原則等課題，累積國內之本土經驗，與國外文獻、臨床經驗相比較，不僅可增進病人用藥安全、達到最大的經濟效益，也能以論文期刊的方式與世界各國分享我國的用藥經驗。

本研究目的是藉由前瞻性的觀察，監測 ART 血中最低濃度（trough），紀錄服藥期間的治療效果（血漿愛滋病毒量、CD4 淋巴球數變化）與療效、副作用的發生率與嚴重度，藉以探索其相關性，不僅可確認國內成立 PK lab 的可行性、監測血中濃度的必要性及適當範圍，ART 血中濃度的結果可提供臨床醫師調整劑量的參考資料。此外，本研究將詢問患者是否併用 ritonavir、任何胃藥、影響 CYP3A4 的藥品/食品/中草藥等，以觀察藥品—藥品或藥品—食品交互作用對 ART 血中濃度的影響。

（二）材料與方法

執行期間： 2015 年 1 月 1 日迄 2015 年 12 月 31 日。

研究方法：

病患收納條件

一、十八歲以上感染愛滋病毒患者，即將開始接受ATV、EFV或NVP等ART或cotrimoxazole, rifabutin治療的愛滋病毒感染者，在填妥同意書後可以加入本研究；

二、病患願意在未來服用抗結核藥物的九到十二個月中繼續在本院持續追蹤治療者。

排除條件

一、已知對於將使用之上述ART過敏或無法耐受者

二、曾經接受過上述ART並且產生抗藥性者

三、臨床醫師判斷患者病況嚴重，無法存活一個月以上

研究步驟

一、針對即將開始接受上述ART治療者，檢測服藥前CD4淋巴球數、血漿愛滋病毒量、肝腎功能指數、血液相檢查、凝血功能檢查。並記錄所有用藥及劑量。

二、開始服用ATV後，一週時檢測血中最低濃度（trough concentration, C24 concentration）或服藥後血中第12 ± 1小時濃度（C12 concentration）；使用EFV者，兩週後檢測服藥後血中第12 ± 1小時濃度（C12 concentration）。使用NVP者，若於lead-in phase時一天服藥一次者，兩週時或於lead-in phase結束前，檢測服藥後血中第24 ± 1小時濃度（trough concentration）；若於maintenance phase時一天服藥兩次者，兩週後檢測服藥後血中第12 ± 1小時濃度（trough concentration）；若病人發生皮疹或肝毒性時，臨床醫師可視情況檢測血中濃度，並記錄於停藥後第幾小時測得。

三、服用四週後，依尋常愛滋病患的醫療，追蹤CD4淋巴球數、血漿愛滋病毒量、肝腎功能、血液相檢查、凝血功能檢查、空腹血醣檢查、血脂肪（三酸甘油脂、LDL、HDL）檢查；相同檢查在後續追蹤當中每十二個星期執行一次，一直到服用抗病毒藥物四十八週為止。

四、住院中每星期接受肝腎功能與血液相、凝血功能檢查。

五、若生化檢驗值顯示異常，將依尋常愛滋病患的醫療，安排相關檢查，並再次檢測ART血中最低濃度。

六、紀錄患者是否併用任何胃藥、影響CYP3A4的藥品/食品/中草藥等。

研究地點

台大醫院愛滋病防治中心專屬愛滋病房及/或門診

ART 血中濃度檢測：

ATV 血中濃度檢測

本研究使用HPLC檢測ATV血中濃度。方法詳述如下：

一、HPLC 系統

a. 儀器：包含自動注射器（autosampler）、梯度幫浦（gradient pump）、層析管柱恆溫箱、紫外線偵測器（UV detector）、電腦設備及分析軟體。

b. 管柱（column）：Mightsil RP-18 GP，with 5 μm beads，4.6 mm-25 cm，並附有相容的保護管柱（guard column）。

c. 移動相（mobile phase）：10 mM 磷酸鹽緩衝溶液(pH=2.5)與 acetonitrile (ACN) 比例為 58:42 (v/v)。

d. 紫外線偵測波長：249 nm。

e. 流速：1 mL/min

f. 注射體積：30 μL

g. 滯留時間（retention time）：ATV 為 13.83 分鐘，diazepam (internal standard) 為 17.23 分鐘。

二、標準品之製備

將 ATV 與 diazepam 溶於 methanol 中，製成 1 mg/mL 的標準品貯液，存放於-20°C；再以 methanol 稀釋成 1.5、5、10、25、50、75、100 μg/mL 的 ATV 工作液與 10 μg/mL 的 diazepam 工作液，存放於 4°C。將工作液加入健康受試者之血漿，使血漿標準檢品中 ATV 濃度分別為 0.15、0.5、1、 2.5、5、7.5、10 ng/mL。

三、血漿檢品前處理

在配製好的血漿標準檢品及病人之待測檢品(各 400 μL)中分別加入 25 μL 的 diazepam、400 μL 2M sodium carbonate 及 800 μL ethyl acetate-n-hexane (50：50 v/v)，經由 vortex 混合均勻，利用高速離心機(轉速為 17900 g)離心，於-80°C 冷凍 30 分鐘，再取出所有有機層溶液，最後以氮氣將有機層溶液吹乾。加入 200 μL methanol 以溶解管內剩餘乾燥物，以孔徑 0.22 μm 的 PVDF filter 過濾，取出其中 30 μL、打入 HPLC 中分析。

四、分析方法之確效

a. 準確度（accuracy）及線性（linearity）

分析一系列的 ATV 血漿標準檢品，濃度由 0.15 到 10 μg/mL。連續 6 天檢測這些血漿標準品，以評估一日內（intra-day）與異日之間（inter-day）濃度檢測變異性。實驗結果顯示濃度自 0.15 到 10 μg/mL 間皆呈線性，準確度為 90.9%～97.8%。

b. 精確度（precision）

將連續 3 天檢測 3 種濃度的 ATV 血漿標準檢品（0.15, 5, 10 μg/mL）以分析精確度。實驗結果顯示精確度為 1.90%～2.55%。

c. 回收率（recovery）

將 ATV 加入無藥（drug-free）血漿中調成 3 種不同濃度的（0.15, 5, 10 μg/mL)、加入 internal standard。ATV 自血漿檢品中的回收率百分比是比較 methanol 萃取前後 peak height ratio 的差異，共執行 3 次。實驗結果顯示回收率為 95%。

d. 選擇性（selectivity）

過去的文獻已評估過一般常與 ATV 併用的藥品並不會干擾本研究將採用的 HPLC 分析方法，包括抗愛滋病毒藥 efavirenz, nevirapine, zidovudine, didanosine, stavudine, lamivudine, indinavir, nelfinavir；抗結核病藥 rifampicin, isoniazid, pyrazinamide, ethambutol, streptomycin；及其他常見併用藥品 ofloxacin, acetazolamide, loperamide, prednisolone, phenytoin, amitriptyline, cotrimoxazole, fluconazole。

e. 定量極限（limit of quantification；LOQ）及偵測極限（limit of detection；LOD）

LOQ：將健康人血漿檢品逐步稀釋至其訊號與雜訊的比值（S/N）等於 10，則此血漿標準品濃度為本分析方法之定量極限。

LOD：將健康人血漿檢品逐步稀釋至其訊號與雜訊的比值（S/N）等於 3，則此血漿標準品濃度為本分析方法之偵測極限。實驗結果顯示 LOQ 為 0.15 μg/mL，LOD 為 0.1 μg/mL。

f. 安定性（stability）

根據過去的文獻，ATV 的血漿溶液可在室溫下保存 4 天、-20°C 下保存一年不變質。

五、病人檢體收集（samples）

由於 ATV 膠囊應與正餐一起服用以提高口服吸收率，病人可能習慣早餐或晚餐時服用，但一般抽血時間大多排定為早晨，因此視病人服藥習慣，待開始治療後的第 8 天之後，於前一天服藥後 12 ± 1 小時或 24 ± 1 小時抽取 7 c.c.的血液分別測定 C12 或最低血中濃度（C24）。血液樣品使用含足夠抗凝血劑 K2EDTA 的小管收集，運送過程中以 4°C 保存。病人全血利用高速離心機 2500 g 在室溫下離心十分鐘，將上清液（血漿）分裝於冷凍小管。取 400 μL 血漿以最適化條件分析病人血中 ATV 的濃度。剩餘之血漿置於-80°C 下保存。

EFV 血中濃度檢測

本研究使用HPLC檢測EFV血中濃度。方法詳述如下：

一、 HPLC 系統

a. 儀器：包含自動注射器（autosampler）、梯度幫浦（gradient pump）、層析管柱恆溫箱、紫外線偵測器（UV detector）、電腦設備及分析軟體。

b. 管柱（column）：Mightsil RP-18 GP，with 5 μm beads，4.6 mm-25 cm，並附有相容的保護管柱（guard column）。

c. 移動相（mobile phase）：10 mM 磷酸鹽緩衝溶液(pH=4)與 acetonitrile (ACN) 比例為 57:43 (v/v)。

d. 紫外線偵測波長：245 nm。

e. 流速：1 mL/min

f. 注射體積：20 μL

g. 滯留時間（retention time）：EFV 為 13.43 分鐘。

二、 標準品之製備

將 EFV 溶於 methanol 中，製成 1 mg/mL 的標準品貯液，存放於 4°C；再以 methanol 稀釋成 100 μg/mL 的 EFV 工作液並加入健康受試者之血漿，使血漿標準檢品中 EFV 濃度分別為 0.5、2.5、5.0、7.5、10.0 μg/mL，以建立定量線。

三、 血漿檢品前處理

在配製好的血漿標準檢品及病人之待測檢品（各 300 μL）中分別加入 300 μL 的 acetonitrile 進行去蛋白步驟，經由 vortex 混合均勻，利用高速離心機（轉速為 17900 g）離心，再取出 400 μL 的上清液，在 40℃下以氮氣將上清液吹乾。最後加入 200 μL 動相以溶解管內乾燥物，以孔徑 0.22 μm 的 PVDF filter 過濾取出其中 20 μL、打入 HPLC 管柱。

四、 分析方法之確效

a. 準確度（accuracy）及線性（linearity）

分析一系列的 EFV 血漿標準檢品，濃度由 0.5 到 10 μg/mL。連續 3 天檢測這些血漿標準品，以評估一日內（intra-day）與異日之間（inter-day）濃度檢測變異性。實驗結果顯示濃度自 0.5 到 10 μg/mL 間皆呈線性，準確度為 97.7% ~ 101.6%。

b. 精確度（precision）

將連續 3 天檢測 3 種濃度的 EFV 血漿標準檢品（0.5, 5.0, 10.0 μg/mL）以分析精確度。實驗結果顯示精確度為 1.15% ~ 1.93%。

c. 回收率（recovery）

將 EFV 加入無藥（drug-free）血漿中調成 3 種不同濃度的（0.5, 5.0, 10.0μg/mL）標準檢品，比較樣品前處理前後 peak area 的差異，共執行 3 次。實驗結果顯示回收率為 101%。

d. 選擇性（selectivity）

需評估一般常與 EFV 併用的藥品是否會干擾本研究將採用的 HPLC 分析方法，包括抗愛滋病毒藥 atazanavir, nevirapine, zidovudine, didanosine, stavudine, lamivudine, indinavir, nelfinavir；抗結核病藥 rifampicin, isoniazid, pyrazinamide, ethambutol, streptomycin；及其他常見併用藥品 ofloxacin, acetazolamide, loperamide, prednisolone, phenytoin, amitriptyline, cotrimoxazole, fluconazole。

e. 定量極限（limit of quantification；LOQ）及偵測極限（limit of detection；LOD）

實驗結果顯示 LOQ 為 0.5 μg/mL，LOD 為 0.2 μg/mL。

五、 病人檢體收集（samples）

由於 EFV 可能導致病人頭暈或精神狀況不佳，因此一般通常建議病人在睡前服用。待開始治療後的第 15 天早晨，於前一天服藥後 12 ± 1 小時抽取 7 c.c. 的血液測定最低血中濃度。血液樣品使用含足夠抗凝血劑 K2EDTA 的小管收集，運送過程中以 4°C 保存。病人全血利用高速離心機 2500 g 在室溫下離心十分鐘，將上清液（血漿）分裝於冷凍小管。取 500 μL 血漿以最適化條件分析病人血中 EFV 的濃度。剩餘之血漿置於-80°C 下保存。

測定 nevirapine 血中濃度

本研究使用 HPLC 檢測 NVP 血中濃度。方法詳述如下：

一、HPLC 系統

a. 儀器：包含自動注射器（autosampler，設定於 4°C）、梯度幫浦（gradient pump）、烤箱（設定於 25°C）、紫外線偵測器（UV detector）、電腦設備及分析軟體。

b. 管柱（column）: Mightysil RP-C18 GP column (150 × 4.6 mm ID; particle size, 5 m)，附有相容的保護管柱。

c. 移動相（mobile phase）： acetonitrile 及 10 mmol/L potassium phosphate (pH 4.0)。動相溶液的比例採用等梯度模式，acetonitrile 的比例 30 %、buffer 70%，維持 10 分鐘。

d. 紫外線偵測波長：250 nm。

e. 流速：1.0 mL/min

f. 注射體積：20 μL

g. 滯留時間（retention time）：NVP 為 6.33 分鐘。

二、標準品之製備

NVP 溶於 methanol 中，配成 1 mg/L 的標準品貯液並儲存於 4℃;。上述的標準品貯液經相同溶液稀釋後，加入健康受試者提供的血漿，配成 5 或 6 種濃度的標準檢品以供建立定量線。由於 NVP 的檢驗範圍是 0.05~15.0 mg/L，可配成 0.05、3.0、6.0、9.0、12.0、15.0 mg/L 的標準檢品。

三、血漿檢品前處理

300 μL 標準檢品或病人檢體之血漿混合 500 μL 的 acetonitrile 後，經由 vortex 一分鐘混合均勻，接著離心 10 分鐘形成分層，取出有機層後以氮氣在 37℃下吹乾。乾燥檢品加入 200 μL 的 methanol，並以 ultrasonic 混合均勻後離心 5 分鐘。最後取出上層的回溶溶液經由 PVDF 0.22μm 薄膜過濾作為樣品進行分析。

四、分析方法之確效

a. 準確度（accuracy）及線性（linearity）

分析一系列的 NVP 血漿標準檢品，濃度由 0.5 到 10 μg/mL。連續 3 天檢測這些血漿標準品，以評估一日內（intra-day）與異日之間（inter-day）濃度檢測變異性。實驗結果顯示濃度自 0.5 到 10 μg/ mL 間皆呈線性，準確度為 100.05% ~ 110.83%。

b. 精確度（precision）

將連續 3 天檢測 3 種濃度的 NVP 血漿標準檢品（0.5, 5.0, 10.0 μg/ mL）以分析精確度。實驗結果顯示精確度為 0.05% ~ 1.83%。

c. 回收率（recovery）

將 NVP 加入無藥（drug-free）血漿中調成 3 種不同濃度的（0.5, 5.0, 10.0 μg/mL）標準檢品，比較樣品前處理前後 peak area 的差異，共執行 3 次。實驗結果顯示回收率為 100.5%。

d. 選擇性（selectivity）

需評估一般常與 NVP 併用的藥物是否會干擾目標藥物的定量，因此個別將各藥品研磨溶至 methanol 中調成 10 μg/mL 濃度，分別為單獨注射以及混合不同種藥品注射觀察使用分析 NVP 條件下在 NVP 出現時間中是否有其他藥物之 peak 在相同時間點干擾。已評估過的藥物包括抗愛滋病毒藥 atazanavir, efavirenz, zidovudine, abacavir, lamivudine, lopinavir/ritonavir, tenofovir, emtricitabine, raltegravir potassium；抗結核病藥 rifampicin, isoniazid, pyrazinamide, ethambutol；及其他常見併用藥品 loperamide, cotrimoxazole, fluconazole, clarithromycin, azithromycin, voriconazole, sulfametoxazol, trimethoprim, ganciclovir, atorvastatin, famotidine 等均不會影響 NVP 的定量。詳見附件一。

e. 定量極限（limit of quantification；LOQ）及偵測極限（limit of detection；LOD）
實驗結果顯示 LOQ 為 0.599 μg/mL，LOD 為 0.098 μg/mL。

f. 安定性（stability）
根據過去的文獻，含 NVP 的病人全血經樣品前處理後，取出的動相溶液可在 20℃下保存 3 週。28

測定 cotrimoxozale 血中濃度

本研究使用 HPLC 檢測 cotrimoxozale 血中濃度。方法詳述如下：以含有抗凝血劑（K2EDTA）收集受試者病人血液約 5-7 mL，使用 2500 rcf 離心 10 分鐘以取得血漿，並存放在-80℃冰箱保存。

將病人血漿檢體 200 μL 加入 200 μL 7% 過氯酸（perchloric acid），以去除血漿中蛋白部分，接著利用高速離心機 15000 g 離心 5 分鐘，取出上清液後，以 0.22 μm 濾膜過濾，最後再以高效能液相層析儀（high-performance liquid chromatography, HPLC）分析 SMX-TMP 濃度。

HPLC 使用管柱為 Luna C18 column, 250x4.6 mm, 5 μm（Phenomenex, Torrance, CA, USA），在室溫下操作。分析條件為：移動相（mobile phase）為氰甲烷（acetonitrile）與 7 mM 磷酸二氫鉀（KH2PO4）以體積比 20:80 所組成。7 mM 磷酸二氫鉀（KH2PO4）使用 10 M 氫氧化鈉（NaOH）調整酸鹼值至 pH=6.5。流速設定為 1 mL/min，最後之沖提液以 UV 230 nm 波長吸收偵測。不須內標準品。SMX 和 TMP 的滯留時間分別為 6.98

分鐘及 9.03 分鐘。

分析方法確效之檢量線（calibration curve）方面，SMX 以 5, 10, 25, 50, 100, 200 μg/mL、TMP 以 0.5, 1, 2.5, 5, 10, 20 μg/mL 六點濃度所建立，分別得迴歸式 Y=26186X + 4101.3，相關係數（correlation coefficient，r2）為 0.9998，及 Y= 56858X – 9193.7，相關係數為 0.9996。兩藥物的偵測極限（lower limit of detection, LOD）為 0.5 g/mL，SMX 和 TMP 之定量極限（lower limit of quantification, LOQ）分別為 5 g/mL 及 1 g/mL。每次濃度測量結果將檢測準確度(accuracy)，將控制在 10% 誤差範圍之內；精確度(precision)則進行一日內重複性（repeatability；intra-day precision）及異日間之再現性（inter-day precision；between-run repeatability），分別控制相對標準差（relative standard deviation，RSD）小於 2%及 5%。

（三）結果

以 HPLC 檢測 ART 與 cotrimoxazole (SMX-TMP)血中濃度方法的開發與確效已在往年的結果報告中呈現，因此不再此贅述。此類分析方法之準確度、一日內跟異日間之藥物精確度（precision）都符合標準。將藥物在低、中、高三種濃度於血漿中做三次冷凍（-80℃）解凍（3-cycled freeze-thaw）之安定性試驗，均有良好的準確度（< 10%）及精確度（< 5%）。

近幾年來之研究計畫專注執行 EFV、ATV 的藥物血中濃度與代謝酵素基因型、治療預後的初步分析；本年度仍繼續協助臨床醫師監測藥物血中濃度，迄今已為 780 位服用 EFV、530 位服用 ATV 與 120 位病人服用 NVP 的病人分析藥物血中濃度與代謝酵素基因型、療效、副作用的相關性。

服用 EFV 者的藥物血中濃度差異性相當大，期中分析 780 位服用 EFV 的愛滋病毒感染者的血液檢體，絕大部分的濃度高於治療指引建議的 1 μg/mL，藥物血中濃度平均值為 3.2 ± 1.6 mcg/mL（0～12.84 μg/mL），但有 2 人完全測不到血中濃度、而 1 人的血中濃度高達 12.84 mcg/mL，遠高於臨床研究與治療指引建議的 1~4 mcg/mL 範圍。

EFV 主要經由肝臟酵素 CYP 2B6 代謝，本研究觀察到 CYP 2B6 為異質接合（G516T）者、同型合子慢代謝者的濃度中位數分別高達 3.47 μg/mL 與 8.78 μg/mL，比正常功能的 CYP 2B6 者的 2.50 μg/mL 高。

由於絕大多數病人的 EFV 血中濃度高於治療指引建議的 1 μg/mL，因此針對使用 EFV 達 6 個月以上、血中濃度高於 2 μg/mL、病毒量低於 200 copies/mL 者，我們近來嘗試將 105 位病人的 EFV 劑量減半、密切追蹤血中濃度與臨床療效與副作用。減半前的 EFV 血中濃度平均為 3.69 mcg/mL（Q1-Q3=2.63-4.36 mcg/mL）、減半後追蹤的 64 病人的血中濃度為 1.96 mcg/mL（Q1-Q3=1.53-2.33 mcg/mL），濃度下降 51.6%（範圍 26-80.6%）。36 位病人有病毒量的追蹤，皆為<20 copies/mL。本研究將持續追蹤成效，若未來愛滋病毒感染者若沒有使用 rifampin，也許 EFV 的劑量可以略降，一方面可以替國家節省醫療費用，一方面可以降低愛滋病毒感染者長期接受抗病毒藥物副作用的發生率。

ATV 血中濃度方面，目前已追蹤的 530 位病人中，全數完成測定 ATV 血中濃度。由於 ATV 只需一天服用一次、食物可增進 ATV 胃腸吸收，建議在正餐後服用，為配合

病人回診抽血時間，因此抽血點共有 2 種：C12 (12 hr)濃度，共計 282 人 (57.3%)；C24 (24 hr, trough)濃度，共計 185 人 (37.6%)；抽血或服藥時間不詳 24 人。

文獻建議 C12 濃度應維持在 0.23 mg/L 以上、C24 濃度應維持在 0.15 mg/L 以上；雖然本研究中 C12 濃度平均值為 1.00 mg/L、C12 濃度平均值為 0.73 mg/L，均高於目標值。但 C12 組中濃度達到目標者只有 85.5%，C24 組更降至 37.6%，只有 64.5%病人的藥物血中濃度高於目標值。由於抽血時間多在清晨，因此應鼓勵病人盡量於晚餐後服用 ATV，以增進胃腸吸收，維持療效並避免產生抗藥性。

ATV 主要經由肝臟酵素 UGT1A1 代謝，分析 492 位服用 ATV 病人的 SNP，帶有變異基因 UGT1A1*28 者共 98 人（19.9%），其中只有 2 位帶有雙股 SNP，其他均為 heterozygous 者。

HPLC 分析方法方面，ATV 的定量極限原為 0.1 mg/L，極接近藥品血中濃度的目標值，經過多次測試，成功將最小檢驗濃度（lower limit of quantification, LOQ）自 0.15 mg/L 降低為 0.1 mg/L，增加分析之靈敏度。另外，nevirapine HPLC 分析方法也已完成開發。而 nevirapine 可以與 efavirenz (EFV)前後上機檢測，有助於加速研究進度。

由於 ATV 的血中濃度可能受 tenofovir (TDF)影響而降低，因此若未使用 ritonavir (RTV)的狀況下，國外文獻中不建議 ATV 與 TDF 同時併用，以免影響療效。故本研究分析無使用 RTV、且病毒量小於 200 copies/mL 的病人中，比較 123 位併用 TDF/lamivudine (TDF-based)與 182 位併用其他 NRTI 類(non-TDF-based)達 6 個月以上的病人，發現有 82.7%的病人雖使用 TDF-based 治療，ATV 血中濃度仍達到目標值；而使用 non-TDF-based 治療的病人，只有 66.7%的 ATV 血中濃度可達到目標值。追蹤 24 週後，各有 3.3%的 TDF-based 病人與 6.6%的 non-TDF-based 病人發生治療失敗（viroloical failure, P=0.31）；追蹤 96 週後，兩組各有 5%與 14%的病毒量反彈回升。原就有較高的病毒量（40-200 copies/mL）與缺乏 ATV 血中濃度者較易治療失敗。而 MDR1 (positions 2677 and 3435)、PXR genotypes (position 63396)、UGT1A1*28 在兩組無顯著差異。此結果已在 101 年 9 月的 Interscience Conference on Antimicrobial Agents and chemotherapy (ICAAC)以壁報發表，manuscript 也完成，投稿審閱中。詳請見圖 1-3。

30 位服用 NVP 的病人中，已檢驗 26 位的 NVP 血中濃度，其中有 18 位(69.2%)是在服藥後 12 或 24 小時抽血。基因型方面，16 人(63.3%)無 CYP2B6 變異、10 人（38.5%）與 2 人（7.7%）分別為異型合子、同型合子變異，比例與 EFV 的病人群不盡相同。分

析 NVP 血中濃度發現，九成病人的藥物血中濃度高於治療指引建議的 3 μg/mL，且個體間差異頗大，NVP 平均值為 6.1 ± 4.1 mg/L，其中 1 人偵測不到，而 1 人濃度高達 17.31 mg/L。過高的血中濃度通常都出現在 maintenance phase。未來將收納更多病人以評估濃度相關副作用（如皮疹、肝炎）、治療效果與 NVP 血中濃度的相關性。初步結果在今年 11 月初的 AASP (Asian Association of School of Pharmacy)年會中以壁報發表。

Cotrimoxazole 方面，於 2014 年 1 月 19 日至 2015 年 6 月 30 日間，前瞻性收案量測血中最低濃度（trough concentration）與最高濃度（peak concentration）。82 位被納入分析的病人中，，年齡中位數為 45 歲（範圍 23-87 歲），82.9%是男性，共有 75 位為治療肺囊蟲肺炎的案例。各有 63 人與 68 人進行 NAT1、NAT2 基因型與藥品血中濃度。不論是 SMX 或者 TMP，血中最高濃度及最低濃度均有良好的線性關係($r>0.9$)。治療期間，AST 及 ALT grade1-3 上升者分別佔總人數之 17.3%，這些病人相較於沒有發生 AST、ALT 上升者有較高的 SMX 血中最高及最低濃度（$p<0.05$）；發生高血鉀（發生率 26.9%）及低血鈉（發生率 5.8%）不良反應的病人有較高的 TMP 血中濃度。另外，發生高血鉀不良反應之病人使用較高的劑量（14.1 vs. 12.3 mg/kg/day, $p=0.0302$）。

Rifabutin 只有一位病人檢測，血中濃度在正常範圍內。

本研究發現 EFV、NVP、ATV 與 cotrimoxazole 的個體間濃度差異性大、且部分病人的藥物血中濃度在建議值之外，有需要進行常規的血中濃度監測；針對 ATV 濃度過低者，應考慮加上 ritonavir 併用以提高 ATV 血中濃度、確保療效，但需密集監測總膽紅素數值，以避免副作用。而 EFV 劑量若依血中濃度修改，可避免副作用並達到較佳的經濟學效益。至於針對部分病人的 NVP 劑量是否需考慮調降，則須累積更多經驗後才能提出具體建議。監測 ART 血中濃度的結果可提供臨床醫師做為調整劑量或換藥的重要參考資料，研究成果不僅可用以確認國內成立 PK lab 的可行性、監測血中濃度的必要性，甚至做為衛生主管機關建議國人使用 ART 劑量時的重要依據。

在本研究室將繼續現行模式，除了提供全國各醫療院所常規監測 ATV、EFV、NVP、rifabutin 與 cotrimoxazole 血中濃度的服務、嘗試開發以 HPLC 方法測定其他 ART，在進行臨床相關研究的同時，亦提供臨床醫師重要的藥品濃度訊息。

（四）討論

本研究延續前年度之研究成果，預定於本年度繼續協助臨床醫師監測 ART 血中濃度，並大量收納使用 EFV、ATV、NVP、rifabutin 與 cotrimoxazole 的病人，著手進行藥品血中濃度與代謝酵素基因型、治療預後、副作用的分析。

ATV 臨床使用時，雖比其他蛋白酶抑制劑較少發生代謝方面的併發症，但因經由肝臟酵素 CYP 3A4 代謝，可能因藥品交互作用而影響藥效；另一肝臟酵素 UGT 1A1 負責 ATV 的排除，ATV 與膽紅素競爭的結果可能造成高膽紅素血症，5%左右的病人更會產生明顯的黃疸症狀。國外研究者發現 ATV 的血中濃度是影響高膽紅素血症的重要原因之一，因此建議血中濃度維持在 0.15 - 0.85 μg/mL 之間。[8-11]另一方面，ATV 時需飯後立即服用、避免與制酸劑或其他胃藥併服。加上目前初步分析發現過高的 ATV 血中濃度與嚴重總膽紅素血症相關，與其他國外文獻的結論相符。因此監測 ATV 血中濃度與避免藥品交互作用實屬必要。

EFV 一般的給藥劑量通常是 600 mg 睡前服用，以避免中樞神經相關的副作用。我們監測 EFV 的血中濃度顯示絕大多數病人的數值均高於 HIV 治療指引建議的 1 μg/mL，且四分之三的人用藥時可兼顧療效達成與副作用的避免。

EFV 主要經由肝臟酵素 CYP 2B6 代謝，其基因多型性在歐美、泰國、日本、印度的研究結果均顯示會顯著影響 EFV 的血中濃度，而本研究觀察國人的追蹤結果也印證了這一點。為避免不良反應的產生，目前在極少數療效佳、EFV 濃度高的病人中，已嘗試將劑量降低成每日 300 mg（半顆）、密切追蹤病毒量與 EFV 血中濃度，觀察是否具有藥物經濟學上的優勢。

以 HPLC 定量 NVP 血中濃度的方式建立後，與文獻的建議濃度相比，接近九成病人的 NVP 血中濃度高於治療指引建議的 3 μg/mL。其中個體差異頗大，而過高的血中濃度通常都發生在 maintenance phase。因目前收案人數少，濃度相關副作用（如皮疹、肝炎）、治療效果與 NVP 血中濃度的相關性仍有待評估。本院於去年引進 NVP 長效藥錠，仿單中提到少數病人可能在糞便中發現藥錠殘骸，應不影響療效。但實際使用後，多位病人提到此現象，因此本研究特別進行血中濃度與臨床療效的探索。9 個月內共追蹤 244 位服用 NVP XR 的病人，其中 49 位 (20.0%)發現糞便中有藥錠殘骸。發現者的平均 CD4 值較無發現者高(612 vs. 495 cells/mm^3, P=0.002)、病毒量相近 (1.55 vs. 1.66 log10 copies/mL, P=0.42)。治療 12 週後，發現者的 12 小時 NVP 血中濃度雖有較低的傾向 (3.55

vs. 5.17 μg/mL, P=0.05)，但平均 CD4 值較無發現者高(626 vs. 547 cells/mm^3, P=0.06)、病毒量較低 (1.39 vs. 1.52 log10 copies/mL, P=0.07)，結果已被 PLoS ONE 雜誌接受。詳請見表一。

（五）結論與建議

ATV、EFV 與 NVP 主要經由酵素 CYP 450 代謝，而酵素活性與 SNP、生理病理狀況、藥品交互作用息息相關，因此每個人的藥物血中濃度變動可能影響療效與副作用。國外文獻中多使用 HPLC 檢測 ART 血中濃度，本計畫藉由前瞻性地以 HPLC 測定 ATV、EFV 與 NVP 血中濃度、檢測相關酵素基因型、追蹤療效與副作用，嘗試以 PK lab 的方式，協助各地的醫療人員監測 ART 血中濃度，同時進行臨床研究。

目前追蹤了 780 位服用 EFV、530 位服用 ATV 與 120 位服用 NVP 的病人，研究結果顯示個體間的藥物血中濃度差異極大，有需要進行常規的血中濃度監測。併用 TDF 似乎並未對 ATV 血中濃度造成顯著影響。而過高的 ATV 血中濃度則會使總膽紅素數值異常增高。

雖然絕大多數病人有 EFV 血中濃度在文獻建議的 1～4 μg/mL 之間，但個體間差異大，主要代謝酵素 CYP 2B6 若異質接合（G516T）者，血中濃度接近建議值的上線，此類病人可考慮調降每日劑量為一般建議劑量的一半，以避免副作用並達到較佳的經濟學效益，目前此研究正在進行中。

服用 NVP 的病人中，九成病人的藥物血中濃度高於治療指引建議的 3 μg/mL，且個體間差異頗大。過高的血中濃度通常都發生在 maintenance phase。將收納更多病人以評估濃度相關副作用（如皮疹、肝炎）、治療效果與 NVP 血中濃度的相關性。

本研究雖發現住院的成人使用 SMX-TMP 的劑量低於仿單建議劑量，但大多數病人可達文獻建議之 TMP 治療濃度，因此推測成人可能不需依照仿單建議的使用劑量即可達到理想濃度。肝毒性和 SMX 濃度有顯著關係；電解質不平衡和藥物濃度與使用劑量之間也有顯著關係。病人血中濃度個體間變異性大，因此，監測藥物血中濃度將有助於避免副作用。由於兩藥之血中最高濃度及最低濃度有良好的線性關係，可利用臨床上較易取得之血中最低濃度去預測最高濃度。Rifabutin 只有一位病人檢測，血中濃度在正常範圍內。

對政策之具體建議:確認國內成立 PK lab 的可行性、監測血中濃度的必要性，ART 血中濃度的結果可提供臨床醫師調整劑量的參考資料，做為國人使用 ART 劑量時的重要依據。例如:使用 EFV600mg 的病患如果體重低於 50 公斤或有服藥副作用者可以建議做藥物濃度檢測，可作為調整其服藥劑量減半或三分之二之參考。

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參、圖表

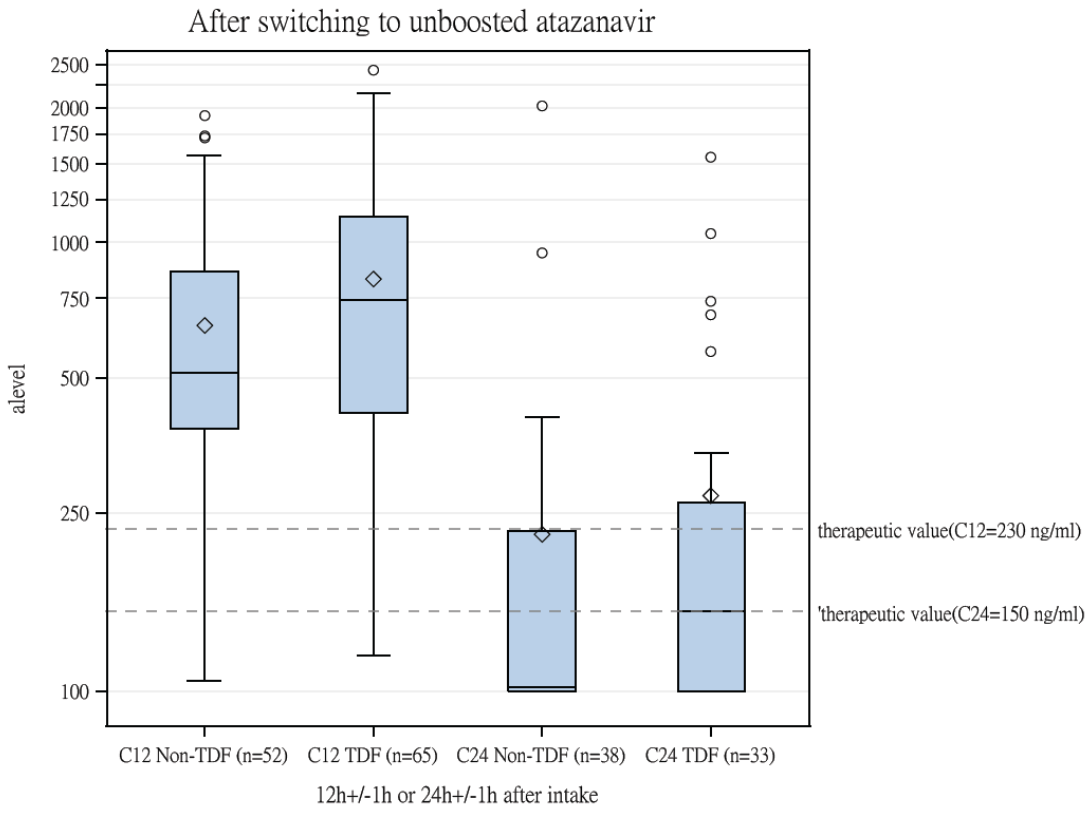


圖 1、Plasma atazanavir concentrations in patients receiving tenofovir, lamivudine plus unboosted atazanavir and those receiving abacavir or zidovudine, lamivudine plus unboosted atazanavir

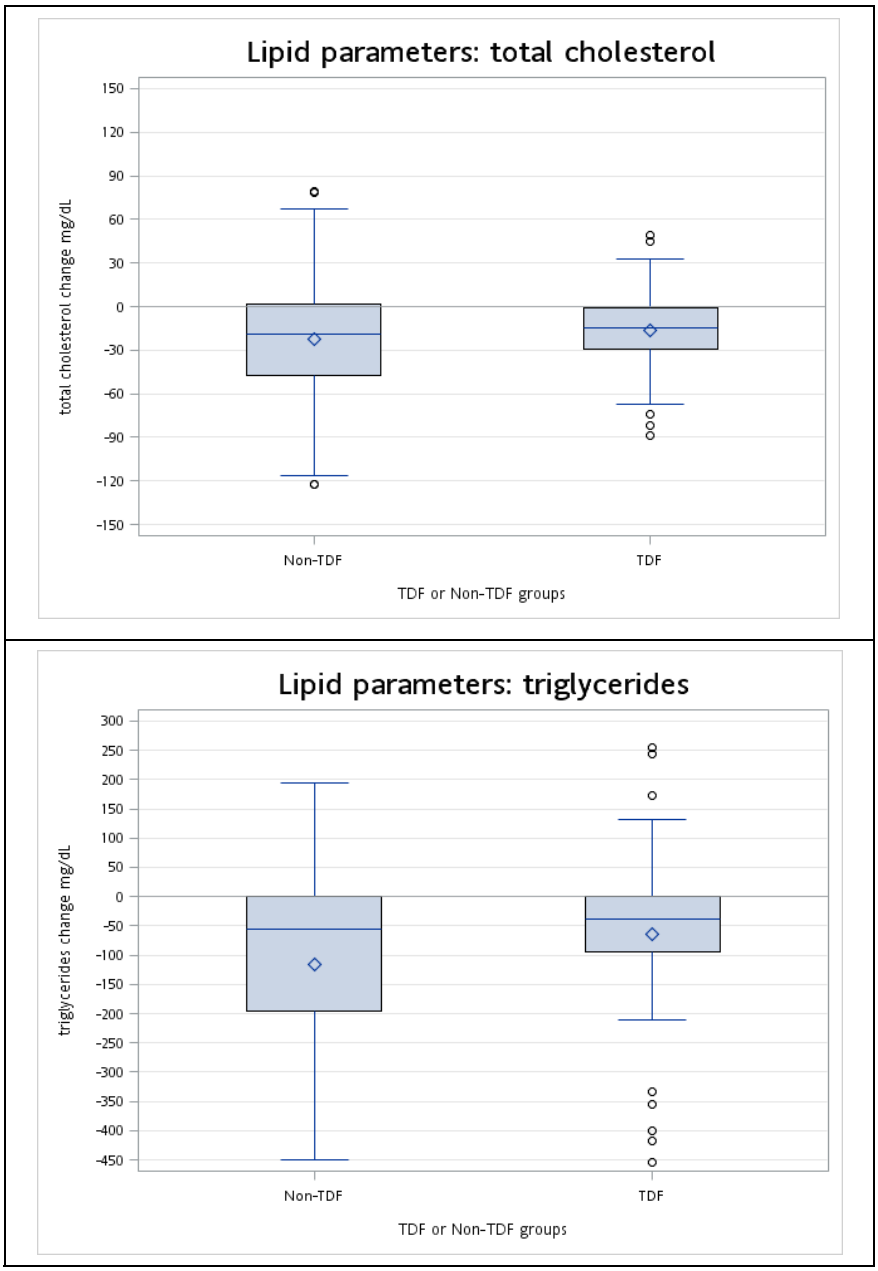


圖 2、Changes of total cholesterol and triglyceride levels before and after switch to unboosted atazanavir plus two nucleoside reverse-transcriptase inhibitors (tenofovir-based vs. non-tenofovir-based)

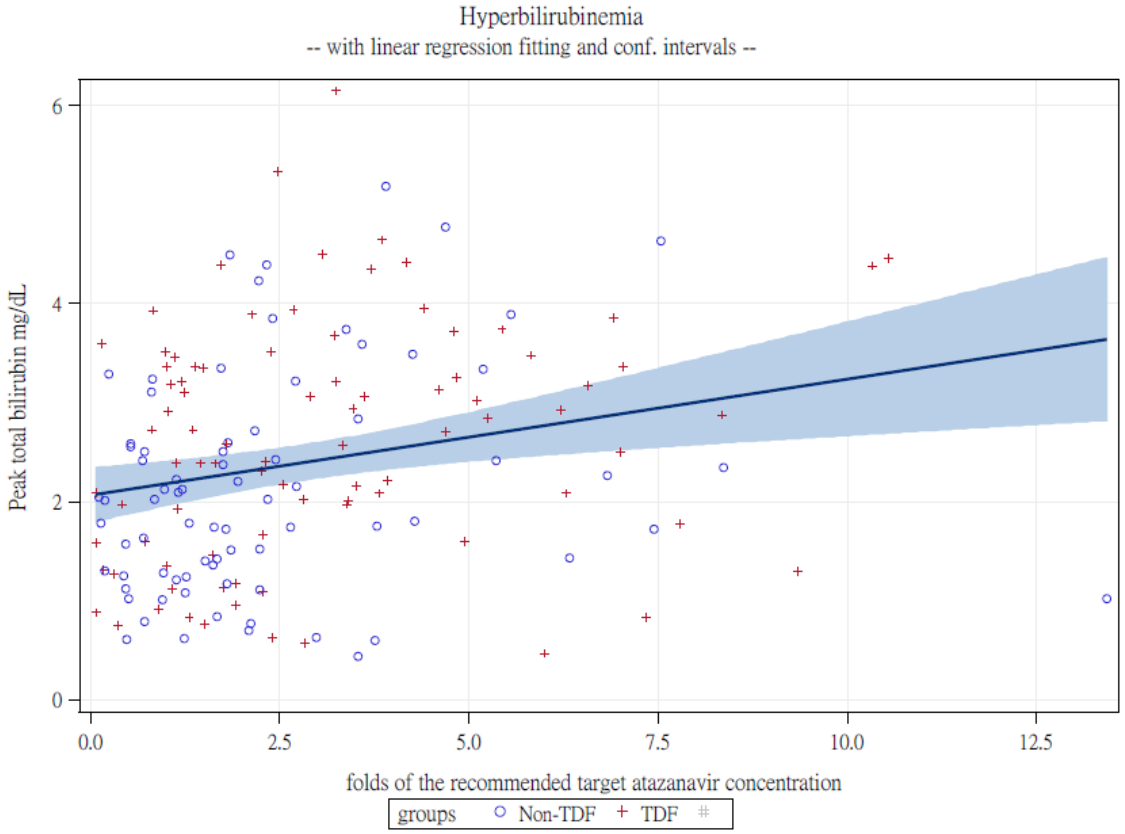


圖 3、Relationship between total bilirubin levels and plasma atazanavir concentrations (expressed in folds above the recommended target atazanavir concentration)

表 1、是否發現糞便中有 NVP 藥錠殘骸者之基本資料與臨床療效比較表

Variables	NVP XR remnant n=49	No remnant n=195	Statistics *P* value
[b]Age, mean ± SD, years	34.4 ± 7.8	38.0 (12.1)	0.01
Male sex	49 (100.0)	186 (94.9)	0.11
Risk for HIV transmission			0.14
MSM/bisexual	47 (95.92)	172 (88.21)	
Heterosexual	1 (2.04)	21 (10.77)	
Others	1 (2.04)	2 (1.03)	
HBsAg-positive	4 (8.16)	13 (6.63)	0.71
Anti-HCV-positive	2 (4.08)	15 (7.65)	0.38
Concurrent medications			
Anti-hypertensives	2 (4.08)	12 (6.12)	0.58
Anti-diabetes	1 (2.04)	5 (2.55)	0.84
Lipid-lowering agents	0	11 (5.61)	0.09
Anti-HCV therapy	0	0	
PVL[c] on switch to NVP XR, mean ± SD, log_{10} copies/ml,	1.55 ± 0.81	1.66 ± 1.03	0.42
CD4 on switch to NVP XR, mean ± SD, cells/mm^3	612.3 ± 272.4	494.9 ± 233.6	0.002
Backbone ARV[d], n (%)			
ABC/3TC	11 (22.45)	62 (31.63)	0.21
TDF/3TC or TDF/FTC	32 (65.31)	107 (54.59)	0.18
AZT/3TC	4 (8.16)	26 (13.27)	0.33
Others	2 (4.08)	1 (0.51)	0.04
Duration of NVP XR before study, mean ± SD, days,	162.1 ± 60.6	154.5 ± 71.7	0.49
First PVL after switch to NVP XR, mean ± SD, log_{10} copies/ml	1.39 ± 0.32 (n=47)	1.52 ± 0.73 (n=173)	0.07
First CD4 after switch to NVP XR, mean ± SD, cells/mm^3	625.9 ± 254.8 (n=46)	546.8 ± 247.8 (n=172)	0.06
Interval between switch and first blood sampling, mean ± SD, days	91.49 ± 46.52	93.06 ± 47.73	0.84

Number of patients undergoing TDM	29 (59.18)	28 (14.29)	<.0001
C_{12}[e] NVP concentrations, median (range), ng/mL,	3.55 (2.5-7.8) (n=20)	5.17 (2.7-12.6) (n=17)	0.05
C_{24}[f] NVP concentrations, median (range), ng/mL,	2.91 (1.2-7.4) (n=9)	3.2 (2.0-6.7) (n=11)	0.98

Note:

a. NVP XR: nevirapine extended release,
b. SD: standard deviation
c. PVL: plasma HIV-1 RNA load
d. ARV: antiretroviral therapy
e. C_{12}: plasma NVP concentration at 12 hours of dosing
f. C_{24}: plasma NVP concentration at 24 hours of dosing

附件五

計畫編號：MOHW104-CDC-C-114-000301

衛生福利部疾病管制署 104 年委託科技研究計畫

計畫名稱：接受合併抗病毒藥物之愛滋病毒感染患者發生低病毒量之多中心

年 度 研 究 報 告

執行機構：台大醫院愛滋病防治中心

計畫主持人：洪健清

研究人員：鄭健禹、羅玉珍、洪健清

執行期間：104 年 1 月 1 日至 104 年 11 月 1 日

＊本研究報告僅供參考，不代表本署意見，如對媒體發布研究成果應事先徵求本署同意*

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壹、中文摘要

低量病毒量的抗藥性的檢測是目前有高度的技術門檻，而隨著病毒量檢測的儀器進步，有越來越多的病人被發現有這個現象。但是該如何處目前尚無定論，所以目前的準則都僅止於專家型的建議。我們進行這一個研究是想探討這個現象的盛行率高低，此外把現有的抗病毒藥物處方如非核苷類反轉錄酶抑制劑(non-nucleoside reverse transcriptase inhibitor；NNRTI)換成蛋不同機轉的蛋白酶抑制劑(protease inhibitors；PI)或是嵌入酶抑制劑(integrase inhibitor)，在我們的觀察中發現在長達一年的追蹤有做處方替換的病人其病毒量可以有較好的控制。在多變相的分析中發現靜脈毒癮者([AOR], 6.81; 95%CI, 2.53-19.87)和病毒量在200-999 copies/mL的病人(AOR, 4.92; 95%CI, 2.01-12.48)有較高的比率被替換處方 (表二)。在48週的觀察中發現，實驗組比對照組有較高比率可以達到HIV病毒量小於40 copies/mL(86.8% vs 66.5%, P=0.011)，但在病毒量小於200 copies/mL的比率兩組間沒有差別(96.6% vs 92.2%, P=0.635)。

關鍵詞：低量病毒量、抗藥性測試、蛋白酶抑制劑、嵌入酶抑制劑、高效能抗病毒組合治療

貳、本文

（一）前言

治療愛滋病毒感染者的準則建議在開始接受高效能抗病毒藥物後愛滋病毒可以達 50 copies/mL 以下，[1] 但是隨著檢驗設備的進步準則修改為病毒量建議達測不到的範圍，此外把病毒量大於 200 copies/mL 定為治療失敗。[2, 3] 檢驗愛滋病毒量的設備不斷更新可以測量的數值，因此在臨床上就有越來越多低病毒量的病人，而血液中的病毒量越低達測不到的狀態就長期來說應該反應的是較好的免疫力和臨床益處，[4, 5] 但是多低的病毒量可以確保病人的免疫力是相對安全的，目前尚無定論。在一篇研究中指出若病人服藥後能早期達到病毒無法測到的狀態，則不管在 CD4 增加的量或是相關死亡率都可以改善。[6]

因此，最近有越來越多的研究在討論在開始接受高效能抗病毒藥物後無法達到病毒量測不到且無法檢驗抗藥性的這一群病人，[7, 8]不管在臨床上或是實驗室裡所代表的意義是甚麼。無法達到測不到愛滋病毒量但持續低病毒量的人可能在未來治療的過程中會有較高比率面臨病毒突變而治療失敗，[8, 9] 因而導致 CD4 淋巴球的增加受到抑制。這當中有相當多的因素會影響愛滋病毒無法達到測不到的範圍，例如:病人服藥順從性、病毒抗藥性程度、藥物血中濃度等。

至於低病毒量該如何處理，目前尚無定論，但不同準則有不同的建議，有些建議把把非核苷類反轉錄酶抑制劑(non-nucleoside reverse transcriptase inhibitor；NNRTI)換成蛋白酶抑制劑(protease inhibitors；PI)，有些建議必須先加強病人服藥的順從性或是做抗藥性測試。[10]

於是，本研究目的旨在推估在服用高效能抗病毒藥物後愛滋病毒量無法達到測不到範圍而持續低病毒量的感染者，是否在未來治療的過程中會面臨較高治療失敗機率或是 CD4 淋巴球的增加會較差。針對這些病人，臨床醫師可會選擇以下三種處理方式: 維持目前治療處方，並加強藥物遵囑性的提醒; 將現有使用含 NNRTI 處方換成 boosted protease inhibitors;或者把目前處方換成 raltegravir 400 mg bid 和 Lopinavir/ritonavir 2 顆 bid 或者是 raltegravir 400 mg bid 和 darunavir (800)/ritonavir (100 mg) qd）。我們將針對這些處理方式的病患觀進行察，並追蹤 24 週和 48 週後檢測病毒可以達到測不到的比率。這一個延續性的研究, 今年第二年, 因此在收案方法和統計模式跟第一年一樣。

（二）材料與方法

1. 研究方法

研究地點：愛滋病指定醫院病房或門診

預計研究時限：103/1/01-104/12/31

研究設計：前瞻性觀察研究

收納條件：

(1) 年齡≥20 歲，初次開始接受高效能抗病毒治療藥物至少六個月之愛滋病毒感染病患，且病毒量（每隔 3 個月檢測）在病人接受高效能抗病毒治療藥物六個月後至少連續兩次無法達到測不到的條件(50-1000 copies/ml)。

(2) 同意繼續在門診追蹤至少一年。

(3) 同意參加本研究。

排除條件：

(1) 目前正在接受伺機性感染治療的病人。

(2) 對治療藥物（Darunavir、Ritonavir、Raltegravir、Kaletra）有嚴重不良反應者。

2. 實驗室檢驗

(1) 於同意參加本研究時，接受病史詢問，病患抽血檢驗 CD4、愛滋病毒量、病毒抗藥性測試、 HBsAg、Anti-HCV。

(2) 針對這些病人，臨床醫師可會選擇以下三種處理方式: 維持目前治療處方，並加強藥物遵囑性的提醒; 將現有使用含 NNRTI 處方換成 boosted protease inhibitors; 或者把目前處方換成 raltegravir 400 mg bid 和 lopinavir/ritonavir 2 顆 bid 或者是 raltegravir 400 mg bid 和 darunavir (800)/ritonavir (100 mg) qd）。我們將針對這些處理方式的病患觀進行察，並追蹤 24 週和 48 週後檢測病毒可以達到測不到的比率。

(3) 於參加本研究後，需每三個月回門診追蹤且持續一年的時間，每次回診需檢驗 CD4 和愛滋病毒量，並詢問病人服用高效能抗病毒藥物的順從性。另外，視臨床狀況增加抽血頻率。

3. 統計分析

研究主持人和協同主持人採用制式的 Excel 紀錄，並將運用 SPSS 統計和分析資料。

（三）結果

截至2015年10月為止，一共有208位病人被收錄此觀察性研究，其中142位(68.3%)為觀察組不作處方上的調整，66位(31.7%)為實驗組，把原有的處方置換成不同機轉之抗病毒藥物 (表一)。這兩組的背景資料差別主要是實驗組有較多的靜脈毒癮的病人(12.3 vs 28.1%)，而當低量病毒量被偵測到時其組別間的病毒量中位數分別為(86 vs 149 copies/mL)，且病毒量小於200 copies/mL的比率分別為(84.0 vs 58.7%)。在觀察組中有42位(29.6%)繼續使用2種 nucleoside reverse-transcriptase inhibitors (NRTIs) 加上1種 nNRTI; 30位(21.1%)使用2 NRTIs加上1種 protease inhibitor; 62位(43.7%)使用2 NRTIs加上 boosted protease inhibitor，而8位(5.6%)病人使用2 NRTIs加上1種 integrase inhibitor。

在實驗組中2位(3.0%)處方被替換成2 NRTIs加上1種 protease inhibitor; 58位(87.9%)被替換成2 NRTIs加上boosted protease inhibitor; 3位(4.5%)被替換成6 NRTIs加上1種 integrase inhibitor，而3位(4.5%)使用boosted protease inhibitor加上1種 integrase inhibitor。

在多變相的分析中發現靜脈毒癮者([AOR], 6.81; 95%CI, 2.53-19.87)和病毒量在200-999 copies/mL的病人(AOR, 4.92; 95%CI, 2.01-12.48)有較高的比率被替換處方 (表二)。在48週的觀察中發現，實驗組比對照組有較高比率可以達到HIV病毒量小於40 copies/mL(86.8% vs 66.5%, P=0.011)，但在病毒量小於200 copies/mL的比率兩組間沒有差別(96.6% vs 92.2%, P=0.635)。

（四）討論

低病毒量的抗藥性的檢測是目前有高度的技術門檻，而隨著病毒量檢測的儀器進步，有越來越多的病人被發現有這個現象。但是該如何處目前尚無定論，所以目前的準則都僅止於專家型的建議。我們進行這一個研究是想探討這個現象的盛行率高低，此外把現有的抗病毒藥物處方如非核苷類反轉錄酶抑制劑(non-nucleoside reverse transcriptase inhibitor；NNRTI)換成蛋不同機轉的蛋白酶抑制劑(protease inhibitors；PI)或是嵌入酶抑制劑(integrase inhibitor)，在我們的觀察中發現在長達一年的追蹤有做處方替換的病人其病毒量可以有較好的控制。但是這無法適用在所有的病人上，因為前提是必須改善某些病人的服藥順從性。此外也必須考慮的病毒的突變多樣化，然而如何克服後者的技術門檻須仰賴未來更多的研究支持和協助。

（五）結論

對政策之具體建議:對於低量病毒量患者應該替換成不同機轉的藥物可以改善其病毒被控制的狀況。

（六）參考文獻

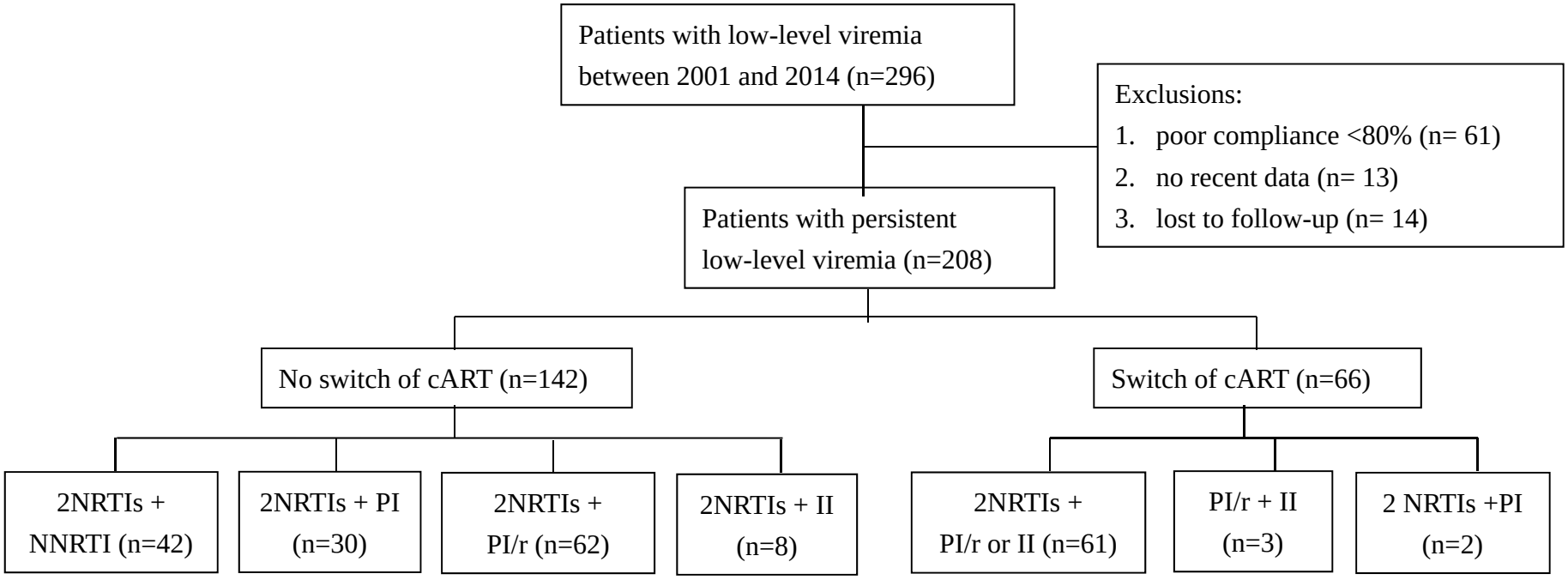
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參、圖表

Figure 1. Between January 2001 and January 2014, patients were enrolled if plasma HIV RNA load (PVL) were >20 to <1000 copies/mL detected for 6 months or more, after having received cART for 6 months or more at two hospitals designated for HIV care in Taiwan.



Multivariate analysis of factors of SWITCH of cART regimen

	P =	Adjusted OR	95% Confidence interval Lower	Upper
Age	0.773	0.995	0.96	1.03
IDU	0.001	6.81	2.52	19.87
PVL at enrollment	0.001	0.996	0.995	0.999
20 – 199 copies/mL	0.001	0.22	0.08	0.53
200 – 999 copies/mL	0.001	4.92	2.01	12.48
Duration of ARV exposure	0.837	0.997	0.993	1.002

附件六

計畫編號：MOHW104-CDC-C-114-000301

衛生福利部疾病管制署 104 年委託科技研究計畫

計畫名稱：愛滋病毒感染患者使用 atazanavir 之抗愛滋病毒組合療法後產生膽結石及腎結石副作用之盛行率及相關影響因子

年 度 研 究 報 告

執行機構：台大醫院

計畫主持人：洪健清

研究人員：王素華、張乃慈

執行期間：104 年 1 月 1 日至 104 年 12 月 31 日

＊本研究報告僅供參考，不代表本署意見，如對媒體發布研究成果應事先徵求本署同意*

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壹、中文摘要

Atazanavir 或 atazanavir 加上 ritonavir 為蛋白酶抑制劑的一種，配合兩種核苷酸反轉錄酶抑制劑，是國際臨床指引中抗愛滋病毒組合療法中建議選用的藥物組合之一。最近於日本所進行的回溯性研究，顯示使用含 atazanavir 藥物組合的病患，相較於使用其他藥物組合，較容易有膽結石及腎結石發生。在本研究中，我們希望了解台灣愛滋病毒感染者發生膽結石及腎結石之盛行率和發生率；並嘗試找出相關影響因子。我們針對 2014 年所有於本院追蹤的愛滋病毒感染者，進行非侵入性腹部超音波檢查；並針對使用 atazanavir 之病患，監測 atazanavir 血中藥物濃度和藥物代謝基因型檢驗。

共有 910 位愛滋病毒感染者接受腹部超音波檢查，其以男性居多(96.9%)，平均年齡為 42 歲，BMI 為 22.9 kg/m^2，已使用抗愛滋病藥物者有 85.8%。其所使用與核苷酸反轉錄酶抑制劑併用之抗愛滋病毒藥物，分別為非核苷酸反轉錄酶抑制劑(49.3%)、atazanavir (34.4%)、lopinavir/ritonavir (20.4%)、及 atazanavir/ ritonavir (5.5%)。膽結石及腎結石的盛行率及粗發生率分別為 18.8%及 7.2%。多變量分析中，與發生膽結石及腎結石相關的因子分別如下：使用 atazanavir/ ritonavir 超過 2 年 (odds ratio [OR], 6.11; 95% confidence interval [CI], 1.05-35.52)、較低的 CD4 基礎值(OR, 1.00; 95% CI, 1.00-1.01)、減低腎絲球濾過率(OR, 1.03; 95% CI, 1.01-1.05)。atazanavir 血中藥物濃度和藥物代謝單核苷酸多型性與膽結石及腎結石並無統計上的相關性。

關鍵詞：膽結石、腎結石、atazanavir、血中濃度監測、藥物代謝單核苷酸多型性

貳、本文

（一）前言

膽結石及腎結石的發生有許多相關因子，包含年齡、性別及其他代謝相關因子 [1-3]。在愛滋病感染者，膽結石及腎結石的盛行率及相關因子目前仍不清楚 [4,5]。過去文獻中，曾經報導與發生膽結石及腎結石相關的藥物包含 indinavir 及 atazanavir [6-9]；然而過去的研究皆有共同的缺點：對於膽結石及腎結石的定義不夠客觀明確、愛滋病感染者接受腹部超音波的原因不同、腹部超音波執行檢查者不同、atazanavir 的血中藥物濃度不清楚、研究結果侷限於日本人種族群等等 [10]。　過去血中藥物濃度和藥物代謝單核苷酸多型性被應用在減少 indinavir 及 atazanavir 的副作用[11-15]；然而，atazanavir 血中藥物濃度和藥物代謝單核苷酸多型性與膽結石及腎結石的關係目前仍不明確。因此在本研究中，我們希望了解台灣愛滋病毒感染者發生膽結石及腎結石之盛行率和發生率；並嘗試找出相關影響因子。

（二）材料與方法

針對 2014 年於本院追蹤的愛滋病毒感染者，使用制式的病患資料 excel 檔案記錄病歷中病患的基本資料(如身高、體重)、過去病史(如糖尿病、肝硬化……)、愛滋病症的相關資料、目前使用藥物、CD4 淋巴球數量、血漿愛滋病毒量、追蹤之實驗數據、使用抗愛滋病毒藥物情形(使用藥物期間以及藥物濃度)。再針對這些愛滋病毒感染者，進行非侵入性腹部超音波檢查。由上述結果可分析出膽結石及腎結石的盛行率及發生率，再藉由觀察及統計上述結果，分析各項變因以找出導致膽結石及腎結石的相關因子。

以下針對各實施方法進行細項及步驟之說明:

1. 受試者納入及排除條件

將納入 2014 年所有於台大醫院追蹤之愛滋病感染者，且年齡必需大於 20 歲；使用之抗愛滋病毒藥物組合不在侷限(可含尚未服用任何抗愛滋病毒藥物的病患) [16]。排除條件包含過去抗愛滋病毒藥物暴露史不明或切除膽囊無法分析膽結石結果者。

2. 資料之蒐集處理評估

採用制式的 Excel 紀錄病人資料。本研究中，對研究對象均事先取得知情同意，以獲得相關病歷資料及進行非侵入性腹部超音波檢查。腹部超音波檢查皆由相同之肝

膽腸胃科醫師操作執行。Atazanavir 之血中藥物濃度藉由高效液相層析法(high-performance liquid chromatography, HPLC)分析 [17-19]，並配合限制片段長度多態性(restriction fragment length polymorphism, RFLP)分析藥物代謝之單核苷酸多型性(single-nucleotide polymorphism, SNP) [20,21]。

3. 統計分析方法

所有的統計分析將利用 SPSS software; version 17.0 (SPSS)進行。類別變數將由 $\chi 2$ 或是費雪精確度檢定(Fisher's exact test)分析；連續變數將由 2-sample t 分析。非類別變數將由 Wilcoxon rank sum test 分析。 P 值小於 0.05 將被認為有統計學上的意義。

（三）結果

截至 2014 年 12 月為止，一共有 910 位愛滋病毒感染者接受腹部超音波檢查。

表一顯示此 910 位愛滋病毒感染者之基本及臨床資料。男性居多(96.9%)，平均年齡為 42 歲，BMI 為 22.9 kg/m^2，已使用抗愛滋病藥物者有 85.8%。其所使用與核苷酸反轉錄酶抑制劑併用之抗愛滋病毒藥物，分別為非核苷酸反轉錄酶抑制劑(49.3%)、atazanavir (34.4%)、lopinavir/ritonavir (20.4%)、及 atazanavir/ ritonavir (5.5%)。

圖一顯示此 910 位愛滋病毒感染者之膽結石及腎結石盛行率。整體膽結石及腎結石為 18.8%；其中膽結石盛行率為 12.5%，腎結石盛行率為 8.2%。未使用及已使用抗愛滋病藥物病患，其膽結石及腎結石盛行率分別為 16.3%及 19.2%，並未達到統計上顯著差異。因膽結石及腎結石造成之臨床症狀(包含膽囊炎、水腎等)的發生人數為 19 人(11.1%)，而其中有 16 人需要接受進一步外科處置，9 人曾經暴露過 atazanavir。

表二顯示單變量及多變量分析膽結石及腎結石相關因子結果。於多變量分析中，與發生膽結石及腎結石相關的因子分別如下：使用 atazanavir/ ritonavir 超過 2 年 (odds ratio [OR], 6.11; 95% confidence interval [CI], 1.05-35.52)、較低的 CD4 基礎值(OR, 1.00; 95% CI, 1.00-1.01)、減低腎絲球濾過率(OR, 1.03; 95% CI, 1.01-1.05)。

圖二顯示 180 位愛滋病毒感染者之 atazanavir 血中藥物濃度。其中 16 位有發生膽結石及腎結石，164 位則沒發生。有發生膽結石及腎結石之病患，相較沒發生之病患，其

投與 atazanavir 12 小時後之血中濃度略高 (0.85 vs. 0.66 mg/L)，但未達統計上顯著差異。

表三顯示有檢測藥物代謝基因型之愛滋病毒感染者的單核苷酸多型性結果。於發生及未發生膽結石及腎結石的病人，其 UGT1A1*28 及 MDR1 2677T/A 的分布是相似的 (UGT1A1*28：12.5% vs. 19.9%；MDR1 2677T/A：71.4% vs. 70.9%)。

（四）討論

先前台灣針對健康族群的盛行率調查，膽結石為 4.6-10.7% [22,23]，腎結石為 7.4% [24,25]。此研究中，我們發現台灣愛滋病感染者，有較高的膽結石及腎結石盛行率。即使是未使用抗愛滋病藥物者，其膽結石及腎結石盛行率也相對較高，其原因可能與收納病人有一半以上為 B 肝及 C 肝感染有關 [26,27]；而是否與愛滋病本身疾病相關，則需要進一步的研究。

膽結石及腎結石的發生有許多相關因子，包含年齡、性別及其他代謝相關因子 [28]。愛滋病感染者在過去文獻中，曾經報導與發生膽結石及腎結石相關的因子包含 indinavir 及 atazanavir [29-32]。此研究中，我們也發現使用 atazanavir/ ritonavir 超過 2 年為膽結石及腎結石之相關因子， 但卻與血中藥物濃度和藥物代謝單核苷酸多型性並無相關性 [33]。我們另外發現較低的 CD4 基礎值也與膽結石及腎結石相關，可能解釋原因為，過去文獻顯示較低的 CD4 與年齡相關併發症(如糖尿病、腎衰竭)有關 [34,35]。

（五）結論

我們的研究顯示台灣愛滋病毒感染者，有較高的膽結石及腎結石盛行率。其相關因子為使用 atazanavir/ ritonavir 超過 2 年、較低的 CD4 基礎值、及減低腎絲球濾過率。但 atazanavir 血中藥物濃度和藥物代謝單核苷酸多型性並無影響。

此研究提供對臨床照護之具體建議為，對於台灣愛滋病毒感染者，若使用 atazanavir/ ritonavir 超過 2 年，應定期追蹤腹部超音波以便提早發現膽結石及腎結石。

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參、圖表

Table 1. The demographic and clinical characteristics of 910 HIV-positive patients who had undergone abdominal sonography with or without cholelithiasis and/or nephrolithiasis .

Variable	All patients[a] (n = 910)	Any stone (n = 70)	No stone (n = 739)	*P*
Age, mean ± SD, years	42.2 ± 10.6	46.0 ± 9.8	41.3 ± 10.5	0.001
Male sex, n (%)	882 (96.9)	65 (92.9)	721 (97.6)	0.042
Homosexual male, n (%)	711 (78.1)	48 (68.6)	587 (79.4)	0.035
Body-mass index, mean ± SD, kg/m^2	22.9 ± 3.5	23.0 ± 3.4	22.9 ± 3.5	0.745
Hepatitis B or C coinfection, n (%)	464 (51.0)	49 (70.0)	384 (52.0)	0.004
Duration of HIV infection, mean ± SD, years,	6.4 ± 5.3	6.6 ± 5.5	6.2 ± 5.2	0.654
Duration of antiretroviral therapy, mean ± SD, years	4.9 ± 4.5	4.9 ± 4.8	4.7 ± 4.3	0.734
Underlying diseases, n (%)				
Hyperlipidemia	185 (20.3)	18 (25.7)	134 (18.1)	0.121
Chronic hepatitis[b]	115 (12.6)	15 (21.4)	87 (11.8)	0.020
Hypertension	99 (10.9)	11 (15.7)	63 (8.5)	0.046
Diabetes mellitus	56 (6.2)	7 (10.0)	36 (4.9)	0.087
Liver cirrhosis	26 (2.9)	4 (5.7)	17 (2.3)	0.100
Chronic kidney disease	20 (2.2)	4 (5.7)	12 (1.6)	0.042
History of antiretroviral therapy, n (%)				
Zidovudine	291 (32.0)	19 (27.1)	226 (30.6)	0.550
Abacavir	422 (46.4)	36 (51.4)	329 (44.5)	0.267
Tenofovir	442 (48.6)	24 (34.3)	368 (49.8)	0.013
Other NRTIs[c]	149 (16.4)	16 (22.9)	108 (14.6)	0.067
NNRTI	449 (49.3)	30 (42.9)	364 (49.3)	0.306
Duration of exposure, mean ± SD, years	2.1 ± 3.3	2.3 ± 3.5	2.1 ± 3.3	0.790
≥ 2 years, n (%)	285 (31.3)	23 (32.9)	230 (31.1)	0.765
Unboosted atazanavir	313 (34.4)	23 (32.9)	243 (32.9)	0.997
Duration of exposure, mean ± SD, years	1.2 ± 2.1	1.0 ± 1.8	1.1 ± 2.2	0.888
≥ 2 years, n (%)	206 (22.6)	14 (20.0)	165 (22.3)	0.654
Atazanavir/ritonavir	50 (5.5)	5 (7.1)	40 (5.4)	0.581
Duration of exposure, mean ± SD, years	0.1 ± 0.6	0.2 ± 0.9	0.1 ± 0.6	0.505
≥ 2 years, n (%)	15 (1.6)	3 (4.3)	10 (1.4)	0.095
Lopinavir/ritonavir	186 (20.4)	11 (15.7)	149 (20.2)	0.372
Duration of exposure, mean ± SD, years	0.8 ± 2.0	0.9 ± 2.3	0.8 ± 1.9	0.496
≥ 2 years, n (%)	133 (14.6)	10 (14.3)	106 (14.3)	0.989
Darunavir/ritonavir	29 (3.2)	1 (1.4)	25 (3.4)	0.719
Duration of exposure, mean ± SD, years	0.1 ± 0.3	0.02 ± 0.2	0.1 ± 0.3	0.377

≥ 2 years, n (%)	10 (1.1)	0 (0)	8 (1.1)	0.999
Indinavir/ritonavir	38 (4.2)	3 (4.3)	25 (3.4)	0.727
Duration of exposure, mean ± SD, years,	0.2 ± 1.3	0.2 ± 1.0	0.2 ± 1.1	0.704
≥ 2 years, n (%)	31 (3.4)	2 (2.9)	21 (2.8)	0.999
Baseline laboratory investigations				
Baseline PVL, median (range), copies/mL	120000 (88-17000000)	228000 (8040-17000000)	111000 (88-10000000)	0.102
Baseline CD4 count, median (range), cells/μL	151 (0-1265)	92 (0-851)	182 (0-1265)	0.025
Follow-up laboratory investigations				
Follow-up PVL, median (range), copies/mL	UD (UD-10000000)	UD (UD-343000)	UD (UD-10000000)	0.026
Follow-up CD4 count, median (range), cells/μL	533 (1-2091)	446 (4-1316)	551 (5-1965)	0.011
Estimated GFR, mean ± SD, mL/min/1.73m^2	99.3 ± 22.7	92.2 ± 25.8	100.5 ± 22.6	0.013
Serum total bilirubin, mean ± SD, mg/dL	1.3 ± 1.7	1.5 ± 2.6	1.2 ± 1.7	0.652
Serum ALT, mean ± SD, U/L	40.6 ± 57.8	58.5 ± 111.0	39.2 ± 53.0	0.053
Serum triglyceride, mean ± SD, mg/dL	157.0 ± 122.9	162.0 ± 94.9	154.8 ± 126.1	0.184
Serum uric acid, mean ± SD, mg/dL	6.0 ± 2.2	6.6 ± 3.3	5.9 ± 2.1	0.755
Urine pH	6.0 ± 0.6	6.0 ± 0.6	6.0 ± 0.6	0.923
Urinary crystal, n (%)	31 (3.4)	4 (5.7)	18 (2.4)	0.086

Abbreviations: ALT, alanine aminotransferase; ARV, antiretroviral; GFR, glomerular filtration rate ; NRTI, nucleoside reverse-transcriptase inhibitor; nNRTI, non-nucleoside reverse-transcriptase inhibitor; PVL, plasma HIV RNA load; SD, standard deviation.

[a]The 910 recruited patients included 70 patients who developed cholelithiasis and/or nephrolithiasis, 739 patients without stone formation, and 101 patients who were excluded from analysis due to no baseline sonographic finding.

[b]Chronic hepatitis was defined as persistent elevation in serum aminotransferase for 6 months or longer.

[c]Other NRTIs included stavudine, didanosine, and zalcitabine.

Table 2. Logistic analysis to estimate the factors associated with cholelithiasis and/or nephrolithiasis.

Variable	Univariate analysis			Multivariate analysis		
	OR	**95% CI**	***P***	**OR**	**95% CI**	***P***
Age, per 1-year increase	1.04	1.02-1.06	0.001	1.03	0.99-1.07	0.187
Male sex	0.33	0.12-0.90	0.031	0.52	0.06-5.01	0.574
Homosexual male	0.57	0.33-0.97	0.037	0.92	0.24-3.55	0.902
BMI, per 1-kg/m^2 increase	1.02	0.94-1.09	0.673	-	-	-
Hepatitis B or C coinfection	2.16	1.27-3.67	0.005	2.09	0.86-5.11	0.105
Duration of HIV infection, per 1-year increase	1.02	0.97-1.06	0.495	-	-	-
Duration of antiretroviral therapy, per 1-year increase	1.01	0.95-1.07	0.779	-	-	-
Underlying diseases						
Hyperlipidemia	1.56	0.89-2.76	0.123	-	-	-
Chronic hepatitis	2.04	1.11-3.77	0.022	0.37	0.04-3.25	0.373
Hypertension	2.00	1.00-4.00	0.050	0.62	0.12-3.31	0.572
Diabetes mellitus	2.17	0.93-5.08	0.074	-	-	0.998
Liver cirrhosis	2.57	0.84-7.87	0.097	3.12	0.28-34.76	0.355
Chronic kidney disease	3.67	1.15-11.70	0.028	2.02	0.27-14.99	0.493
History of antiretroviral therapy						
Zidovudine	0.85	0.49-1.47	0.550	-	-	-
Abacavir	1.32	0.81-2.16	0.268	-	-	-
Tenofovir	0.53	0.32-0.88	0.014	0.95	0.34-2.65	0.920
Other NRTIs	1.73	0.96-3.14	0.070	1.04	0.29-3.78	0.956
NNRTI, ≥ 2 years	1.08	0.64-1.83	0.765	-	-	-

Unboosted atazanavir, ≥ 2 years	0.87	0.47-1.60	0.654	-	-	-
Atazanavir/ritonavir, ≥ 2 years	3.26	0.88-12.15	0.078	6.11	1.05-35.52	0.044
Lopinavir/ritonavir, ≥ 2 years	1.00	0.49-2.01	0.989	-	-	-
Darunavir/ritonavir, ≥ 2 years	-	-	0.999	-	-	-
Indinavir/ritonavir, ≥ 2 years	1.01	0.23-4.38	0.994	-	-	-
Laboratory investigations						
Baseline PVL, per 1 copies/mL increase	1.00	1.00-1.00	0.030	1.00	1.00-1.00	0.319
Baseline CD4 count, per 1-cell/μL decrease	1.00	1.00-1.00	0.025	1.00	1.00-1.01	0.030
Follow-up PVL, per 1 copies/mL increase	1.00	1.00-1.00	0.750	-	-	-
Follow-up CD4 count, per 1-cell/μL decrease	1.00	1.00-1.00	0.012	1.00	1.00-1.00	0.930
Estimated GFR, per 1-mL/min/1.73m^2 decrease	1.02	1.00-1.03	0.007	1.03	1.01-1.05	0.001
Serum total bilirubin, per 1-mg/dL increase	1.06	0.96-1.17	0.251	-	-	-
Serum ALT, per 1-U/L increase	1.00	1.00-1.01	0.024	1.00	0.99-1.02	0.787
Serum triglyceride, per 1-mg/dL increase	1.00	1.00-1.00	0.648	-	-	-
Serum uric acid, per 1-mg/dL increase	1.14	0.85-1.53	0.388	-	-	-
Urine pH, per 1-unit increase	0.98	0.58-1.66	0.939	-	-	-
Urinary crystal	2.76	0.89-8.55	0.078	-	-	0.999

Abbreviations: ALT, alanine aminotransferase; ARV, antiretroviral; BMI, body mass index; CI, confidence interval; GFR, glomerular filtration rate; NRTI, nucleoside reverse-transcriptase inhibitor; nNRTI, non-nucleoside reverse-transcriptase inhibitor; OR, odds ratio; PVL, plasma HIV RNA load.

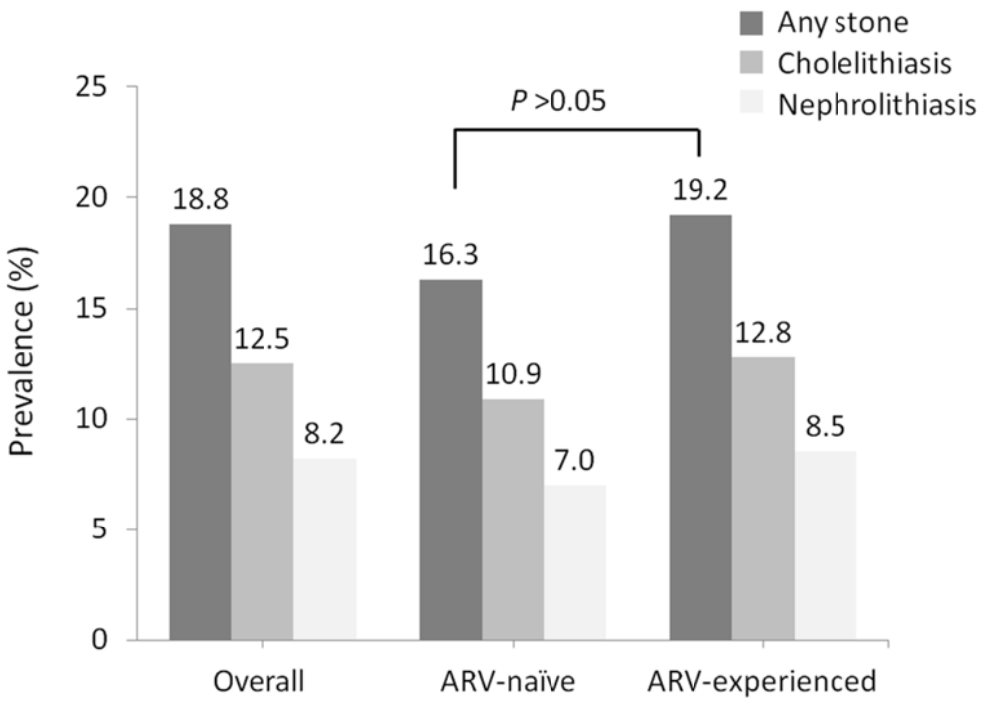
Table 3. Comparisons of genotype frequencies for UGT1A1 and MDR1 2677 between patients with and those without cholelithiasis and/or nephrolithiasis.

Genotype	Any stone n/N (%)[a]	No stone n/N (%)[a]	*P*
UGT1A1*28			
TA6/TA6	14/16 (87.5)	129/161 (80.1)	0.740
TA6/TA7	2/16 (12.5)	32/161 (19.9)	
MDR1 2677			
G/G	2/7 (33.3)	25/86 (29.1)	0.987
G/T	2/7 (33.3)	29/86 (33.7)	
T/T	1/7 (16.7)	12/86 (14.0)	
T/A	1/7 (16.7)	6/86 (7.0)	
G/A	1/7 (16.7)	13/86 (15.1)	
A/A	0/7 (0)	1/86 (1.2)	
MDR1 2677 (T or A)	5/7 (71.4)	61/86 (70.9)	0.999

Abbreviations: MDR1, multidrug resistance gene 1; UGT, UDP-glucuronosyltransferase.

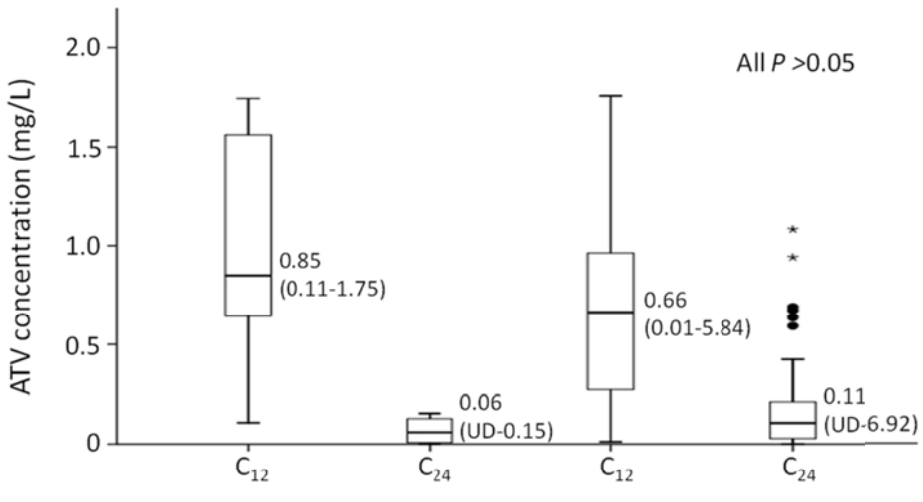
[a]N was the total number of patients with determinations of UGT1A1*28 or MDR1 2677 polymorphisms, and n was the number of patients with specific genotypes.

Figure 1. The prevalence of cholelithiasis and/or nephrolithiasis in 910 HIV-positive patients who had undergone abdominal sonography.



Abbreviation: ARV, antiretroviral.

Figure 2. Plasma atazanavir concentrations of patients with (n = 16) and without (n = 164) cholelithiasis and/or nephrolithiasis.



Patients with TDM	12	4	93	71
ATV >0.85 mg/L, n (%)	6 (50.0)	0 (0)	31 (33.3)	3 (4.2)

Abbreviations: ATV, atazanavir; TDM, therapeutic drug monitoring.

附件七

計畫編號：MOH104-CDC-C-114-000301

行政院衛生署疾病管制局一〇四年度科技研究發展計畫

期末報告

研究名稱：抗人類免疫缺乏病毒藥品處方使用規範效果分析

英文題目：Treatment outcome and effectiveness of different highly active antiretroviral therapy regimens in Taiwan

執行機構：台大醫院愛滋病防治中心

研究人員：洪健清、蔡茂松、張君俞、巫沛瑩、劉玟君、楊上平、廖敏伶

執行期間： 102 年 1 月 1 日至 104 年 10 月 31 日

＊本研究報告僅供參考，不代表本署意見，如對媒體發布研究成果應事先徵求本署同意*

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壹、中文摘要

後天免疫缺乏症候群自從 1981 年在美國發現以來，已成為全世界二十一世紀最重要的公共衛生問題，根據我國衛生署的統計資料顯示，截至 2012 年八月全台灣本國籍 HIV 累積感染人數已經達到 23,474 人，存活者有 19,817 人，感染者存活時間延長是導致累積存活人數快速增加的主因，有效的雞尾酒療法(combination antiretroviral therapy, cART)更是其中的關鍵因素，藥物治療大幅地降低了病患發生伺機性感染（opportunistic infections）和腫瘤的機會與致死的風險。

我國自 1988 年起，政府預算提供感染者免費藥物治療， 1998 年起則由健保局依重大傷病給付，2006 年起愛滋感染者的治療及藥費，改由衛生署疾病管制局每年編列公務預算支應。 依據中央健保局統計，2000 年愛滋感染者醫療費用為四億五千多萬元， 2010 年全年愛滋經費支出大幅增加至 22 億。

因此，疾病管制局訂定「抗人類免疫缺乏病毒藥品處方使用規範」，並公告自 101 年 6 月 1 日起實施，以同療效但價格相對較低的處方作為優先選擇，對於 101 年 6 月 1 日後首次服藥病患，應優先開立第一類處方；若個案因生理因素不適用第一類處方者，才選擇第二或第三類處方。第二類處方或第三類處方之使用。若需使用每月藥價超過 20,500 元之第四類處方者，則必須向疾病管制局申請事前審查。

考慮治療效果、藥物副作用發生率與嚴重程度、抗藥性的型態，此一規範是否能達到無損病人的用藥安全與效果目前仍尚待研究。

關鍵詞：愛滋病毒感染、抗愛滋病毒藥物、副作用、成效分析

貳、本文

（一）前言

後天免疫缺乏症候群（愛滋病，Acquired Immunodeficiency Syndrome, AIDS），自從 1981 年在美國發現以來，已成為全世界二十一世紀最重要的公共衛生問題，依據聯合國愛滋病組織(Joint United Nations Programme on HIV/AIDS; UNAIDS)的資料發現雖然新增愛滋病毒(human immunodeficiency virus, HIV)感染人數似乎有緩慢下降的趨勢，愛滋病死亡的人數在 2005 年達到高峰亦逐漸下降，然而愛滋病毒感染者的存活人數正逐年增加中，2010 年估算約有 3,400 萬人存活，相較於 2001 年的統計增加了 17%，根據我國衛生署的統計資料顯示，截至 2012 年八月全台灣本國籍 HIV 累積感染人數已經達到 23,474 人，存活者有 19,817 人，感染者存活時間延長是導致累積存活人數快速增加的主因，有效的雞尾酒療法(combination antiretroviral therapy, cART)更是其中的關鍵因素，藥物治療大幅地降低了病患發生伺機性感染（opportunistic infections）和腫瘤的機會與致死的風險[1, 2]。

我國自 1988 年起，政府預算提供感染者免費藥物治療，1997 年 4 月開始提供感染者免費雞尾酒療法，1998 年起則由健保局依重大傷病給付，2006 年起愛滋感染者的治療及藥費，改由衛生署疾病管制局每年編列公務預算支應，愛滋病毒感染對於接受治療的病患來說，已不是 20 世紀的黑死病，目前長則二十幾年的存活時間使得愛滋病儼然為一慢性疾病。 依據中央健保局統計，2000 年愛滋感染者醫療費用為四億五千多萬元，每位感染者平均花掉一年 35 萬元藥費，為一般民眾醫療花費的 100 倍，在 2010 年全年愛滋經費支出更大幅增加至 22 億。

由於公務預算有限且醫療費用呈現嚴重赤字，為以有限的公務預算讓所有的感染者都能得到最基本的醫療照顧的權益，疾病管制局訂定「抗人類免疫缺乏病毒藥品處方使用規範」，並公告自 101 年 6 月 1 日起實施，以同療效但價格相對較低的處方作為優先選擇，對於 101 年 6 月 1 日後首次服藥病患，應優先開立第一類處方；若個案因生理因素不適用第一類處方者，才選擇第二(例如不宜使用 zidovudine 者）或第三類處方(例如不宜使用非核苷酸反轉錄酶抑制劑者)。第二類處方或第三類處方之使用。若前三類處方均不適用，而需使用每月藥價超過 20,500 元之第四類處方者，則必須向疾病管制局申請事前審查 (表一)。

在英國已經有研究證實比較不同種類的藥物搭配做為第一線治療，核苷酸反轉錄酶抑制劑加上非核苷酸反轉錄酶抑制劑相較於核苷酸反轉錄酶抑制劑加上蛋白質酶抑制劑，的確能達到降低醫療支出的目的[3]；但也有研究指出在考慮抗藥性及治療失敗因素下，使用較昂貴的核苷酸反轉錄酶抑制劑 tenofovir 相較於舊藥 zidovudine 做為第一線其實是較合乎成本效益的治療選擇[4]；在南非的成本效益研究認為在選擇核苷酸反轉錄酶抑制劑上，應避免 starvudine，至於 tenofovir 與 zidovudine 的比較，雖然治療效果上 tenofovir 優於 zidovudine，但費用上以 zidovudine 較為低廉[5]；美國的研究發現，以成本效益的觀點 abacavir 的第一線治療上可以作為 tenofovir 的替代選擇[6]。

藥物成本效益分析的結果，有部份決定於所鍵入的參數設定，如藥物費用、副作用發生率、抗藥性的比率、更動藥物的比率、治療失敗的發生率及勞動成本等眾多因素，在台灣醫療成本、藥物規範、副作用發生率、抗藥性的型態與英國、美國或其他醫療資源有限的地區均有差異，國外的成本效益分析未必適用於台灣，本研究欲提供實施「抗人類免疫缺乏病毒藥品處方使用規範」後首次服藥病患的臨床資料，作為未來分析的依據。

表一、各類處方之使用規範詳如下表：101 年 6 月 1 日公告

類別	處方(藥物品項)	每月藥價	使用規範
一	Combivir+NVP	15,324	無藥物禁忌症之首次服藥病患優先處方。
	Combivir+EFV	16,992	
二	Viread＋3TC＋NVP	17,262	醫師應於病歷記載使用該類處方之適應症或不宜使用 AZT 之原因。
	Viread ＋3TC＋EFV	18,564	
	Kivexa＋NVP	18,930	
	Kivexa＋EFV	20,232	
三	Combivir+Kaletra	19,550	醫師應於病歷記載使用該類處方之適應症或不宜使用 NNRTI 之原因。
	Combivir+Prezista/r	19,551	
	Combivir+ATV(300)/r	19,551	
	Combivir+ATV(400)	19,554	

四	常見之每月超過 20,500 元之處方如下：		須事前審查。（公告日前已使用該類處方者，需提報備查；公告日後之新使用者，需經審查通過才能使用。）
	Viread+3TC+Kaletra	21,488	
	Viread+3TC+Prezista/r	21,489	
	Viread+3TC+ATV(300)/r	21,489	
	Viread+3TC+ATV(400) 【註 4】	21,492	
	Combivir+Isentress	22,212	
	Kivexa+kaletra	22,790	
	Kivexa+Prezista/r	22,791	
	Kivexa+ATV(300)/r	22,791	
	Kivexa+ATV(400)	22,794	
	Viread+3TC+Isentress	24,150	
	Kivexa+Isentress	25,452	

（二）材料與方法

1. 研究設計: 多中心觀察性研究
2. 研究對象: 101 年 6 月 1 日後首次服藥病患
   (1) 納入條件: 大於十八歲以上於感染科門診追蹤之 HIV 感染患者
   (2) 排除條件: 已知懷孕或即將懷孕的婦女、肝硬化、癌症、慢性腎臟病患、多發性硬化患者、懷孕女性、使用類固醇或免疫抑制劑之患者及過去曾經因為任何原因使用雞尾酒藥物者
3. 資料收集
   (1) 詢問並紀錄病患系統性疾病病史、因任何疾病住院治療、疫苗注射史、抽菸、飲酒習慣、接受其他藥物治療或使用其他藥品或物質
   (2) 我們也會調閱病患的病歷資料
   (3) 病患將依據國內外治療指引，建議於基礎值、用藥後兩周、四到六周與之後每三到六個月接受抽血檢驗，建議檢驗項目如下表

建議血液檢查及時程

	基礎值	二周	四到六周	八周	三個月	六個月
genotypic drug resistance testing	V					
CD4	V		V		V	V
Complete blood count with differential	V	V	V	V	V	V
Plasma viral load	V		V		V	V
Serum liver enzymes: AST ALT	V	V	V		V	V
Blood urea nitrogen/creatinine	V	V			V	V
Serologic tests for syphilis: VDRL/RPR, TPHA	V				V	V
Anti-HAV	V					
HBsAg, anti-HBs, HBc	V					
HCV antibody test (e.g., EIA/ELISA)	V					V
Lipid profile	V					V

(4) 紀錄病患抽血檢查的結果

(5) 紀錄病患使用雞尾酒藥物發生過敏反應的時間與嚴重程度

(6) 紀錄病患使用雞尾酒藥物發生肝炎的時間與嚴重程度

(7) 紀錄病患發生雞尾酒藥物其他相關副作用的時間與嚴重程度

(8) 紀錄病患因各種原因需更換初始雞尾酒藥物的時間及更換後的藥物種類

4. 資料分析

所有的資料收集後，以 Excel 應用程式彙整完成後，以統計軟體 SAS 9.2 版本，先以描述性統計方法分析樣本的基本資料。之後進行卡方檢定(Chi-square)分析，比較不同藥物組合下，影響的各樣結果的因子，採雙尾檢定 p 值＜0.05 為具有統計學上的意義，以多元迴歸邏輯(multiple logistic

regression)中逐步回歸的方法找出有顯著差異的變項，並以統計方法中的殘差分析、影響力分析、以及多元共線性分析去檢定統計結果的正確性。

（三）結果

自 2012 年 6 月(實施抗人類免疫缺乏病毒藥品處方使用規範)後，目前總共陸續收集 965 位 HIV 病毒感染者開始使用抗人類免疫缺乏病毒藥物，其中 97.3%為男性，年齡中位數[median (IQR)]為 30.5(25.9-36.9)歲，開始接受抗病毒藥物治療前，平均 CD4 淋巴球數[median (IQR)]為 288.8 (145.6-398.1) cells/ul，平均病毒量[median (IQR)]為 4.83 (4.43-5.27) log10 copies/mL。

965 位使用抗病毒藥物病患中 35.2%(340)使用第一類處方，58.3%(563)使用第二類處方，3.0%(29)使用第三類處方，3.4%(33)需使用第四類處方。

965 位使用抗病毒藥物病患中 65.0%(628)會至少更動一次處方，開始使用到更動處方時間間隔為[median (IQR)] 35(14-196)天，其中 20.2%(127)是因為臨床懷疑是藥物過敏相關的皮疹，13.9%(87)因為抗病毒藥物相關神經精神副作用而換藥，9.1%(57)產生無法忍受的腸胃道不適而更替藥物，10.8%(68)的病患因為血紅素下降/白血球低下而換藥，值得注意的是 10.4% (65)因為治療效果不如預期或是藥物的抗藥性(genotypic resistance)而改變處方，30.3%(190)則因為服藥方便性(藥物顆粒數和服藥次數)更替處方。

使用卡貝茲(combivir, zidovudine+ lamivudine)超過 28 天的病患，平均血紅素相較於基礎值下降 1.5 g/dL (95% CI. 0.9~2.1)達統計學上的意義。

扣除單純因為服藥方便性而更動藥物者(N=438)，起始處方的選擇與之後持續處方具有統計的相關性，使用第一類處方之後有 65.8%需要更動處方，使用非第一類處方作為起始處方之後有 32.7%需要更動處方，有統計學上顯著的差異(Log-Rank p <0.001)；分為四類發現，使用第一類處方作為起始處方之後有 65.8%需要更動處方，使用第二類處方作為起始處方之後有 32.5%需要更動處方，使用非第三類處方作為起始處方之後有 65.4%需要更動處方，使用第四類處方作為起始處方之後有 36.4%需要更動處方，四組之間有統計學上顯著的差異(Log-Rank p <0.001)。

（四）結論與建議

研究結果顯示，考慮治療效果、藥物副作用發生率與嚴重程度、抗藥性的型態，此一規範對於病人的用藥安全效果、臨床治療效果、符合病患服藥的方便性及順從性等可能有其他更好的方式可以調整。

病患對於藥物組合的臨床耐受性有所不同，參考 WHO 於 2013 年的治療建議及 2015 訂定的治療目標，Combivir 仍做為國內的治療優先選擇必須配合臨床工作人員注意相關的副作用，而一日兩次的服藥限制對於病患的服藥耐受性是一大考驗，長期的副作用則有待日後更長時間追蹤及更多的病患納入分析。

此規範為 2012 年中開始實施，WHO 於 2013 年更改治療指引，而近年來有越來越多的研究開始考慮簡化藥物的方式，除了減少 3 種藥物的可能副作用外，減少藥物種類的另一項好處是減少藥物的費用，而未來的 1-2 年內許多藥物的專利期即將終止，面對學名藥的競爭，藥物的價格可望大幅下降，我國未來的治療指引，在治療選擇與藥物價格的抉擇中，將更具彈性。

對政策之具體建議:期待新的處方能以實證醫學為基礎，提高台灣愛滋病毒感染者的醫療品質。

TIME'S UP
Patents on several key HIV drugs are set to expire over the next five years

Drug	Type	Manufacturer	Expiry
3TC (lamivudine)	Nucleoside reverse transcriptase inhibitor	GlaxoSmithKline	2010
Abacavir	Nucleoside reverse transcriptase inhibitor	GlaxoSmithKline	2012
Efavirenz*	Non-nucleoside reverse transcriptase inhibitor	Bristol-Myers Squibb	2013
Delavirdine	Non-nucleoside reverse transcriptase inhibitor	Pfizer	2013
Darunavir	Protease inhibitor	Tibotec	2015
FTC (emtricitabine)*	Nucleoside reverse transcriptase inhibitor	Gilead	2015
Tipranavir	Protease inhibitor	Boehringer Ingelheim	2015
Ritonavir	Protease inhibitor	Abbott	2016
Tenofovir*	Nucleotide reverse transcriptase inhibitor	Gilead	2017

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附件八

計畫編號：MOHW104-CDC-C-114-000301

衛生福利部疾病管制署104年委託科技研究計畫

期末成果報告

研究名稱：合併HIV與HBV感染者使用tenofovir治療之長期追蹤研究

英文題目：Long-term virological outcome of HBV infection in HIV-infected patients receiving TDF/3TC-containing HAART

執行機構：台大醫院愛滋病防治中心

研究人員：李官燁、洪健清

執行期間： 104 年 1 月 1 日至 104 年 12 月 31 日

＊本研究報告僅供參考，不代表本署意見，如對媒體發布研究成果應事先徵求本署同意*

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壹、摘要

（一）中文摘要

台灣是 HBV 高流行地區，HIV 感染者之慢性 HBs Ag 帶原率約為 16%。Lamivudine 可同時抑制 HIV 與 HBV 的複製，然而使用 lamivudine 治療 HIV 感染者之 HBV 每年約有 12%產生 YMDD 抗藥性。Tenofovir 也是一種可同時抑制 HIV 與 HBV 複製的核苷酸反轉錄酶抑制劑，早期使用在 HIV 合併 HBV 感染者當發生 lamivudine 抗藥性突變時，作為第二線的抗 HBV 藥物；自 2011 年 6 月開始，tenofovir 也納入為 HIV 感染者第一線的抗病毒藥物選擇之一。

本研究旨在監測分析 HIV 合併 HBV 感染者在使用 tenofovir 治療後，其 HBV 相關之臨床與病毒學療效，探討有無 lamivudine 抗藥性突變與 tenofovir 療效之關係，與傳統使用 lamivudine 治療之病患對照組比較；以及追蹤長期使用 tenofovir 治療之下，是否會造成 tenofovir 抗藥性突變之發生。

自 2010 年 11 月至 2015 年 10 月，針對具有 HBV lamivudine 抗藥性突變之 HIV/HBV 合併感染者共納入 33 位病患，平均追蹤 3.7 年；第一線使用 tenofovir 治療之 HIV/HBV 合併感染者則共納入 56 位病患，平均追蹤 2.4 年。兩組幾乎皆為男性 (100%及 98.2%)，帶有 lamivudine 抗藥性突變者平均年齡較大 (42±8 vs. 36±8 歲)，兩組病患 HBV 之基因型主要皆為 genotype B (84.8%及 83.9%)，且開始使用 tenofovir 治療前血中 HBV DNA 平均值 (6.1±2.1 vs. 6.0±2.2 copies/mL) 及 HBeAg 陽性率 (48.5% vs. 31.5%, P =0.17) 並無差異。兩組各有 33 位及 52 位病患完成至少一年 (48 週) 的治療與追蹤，其中各有 75.8%及 86.5%的病患其血中 HBV DNA 已降至<128 copies/mL (測不到)。在多變量分析中，惟有 HBeAg 陽性與經 48 週治療後血清仍可測得 HBV DNA 相關 (odds ratio, 11.36, 95% CI, 1.93-66.82, P =0.007)。目前未檢測到 tenofovir 相關之抗藥性突變。

研究結果顯示 tenofovir 對於帶有 lamivudine 抗藥性突變，及無 lamivudine 抗藥、第一線使用抗病毒藥物治療之 HIV/HBV 合併感染者，其治療效果一樣好。

關鍵詞：B 型肝炎病毒、愛滋病毒、lamivudine 抗藥性

（二）英文摘要

Objectives: Addition of tenofovir is often needed for HIV/HBV-coinfected patients in whom HBV resistance to lamivudine emerges after long-term combination antiretroviral therapy (cART) that contains only lamivudine for HBV. We aimed to evaluate the virological response after switch to tenofovir/lamivudine-containing cART in HIV/HBV-coinfected patients with HBV resistance to lamivudine.

Methods: Between November 2010 and October 2015, two groups of HIV/HBV-coinfected patients were enrolled: cART-experienced patients with HBV resistance to lamivudine after long-term lamivudine-containing cART (lamivudine-resistant group); and cART-naive patients without lamivudine resistance (lamivudine-susceptible group). Serial blood samples were collected for determinations of plasma HBV DNA load every 12 weeks for 48 weeks, and subsequently annually for 5 years.

Results: Thirty-three and 56 HIV/HBV-coinfected patients were enrolled in the lamivudine-resistant and lamivudine-susceptible groups, respectively. Patients in the lamivudine-resistant group were significantly older (mean age, 42 vs. 36 years). The two groups of patients had similar plasma HBV DNA load (mean, 6.1 vs. 6.0 log10 copies/mL) before starting tenofovir/lamivudine-containing cART. The proportion of HBeAg-positive patients was 48.5% and 31.5% in the two groups, respectively (P=0.17). After a mean follow-up duration of 3.7 and 2.4 years, 33 and 52 patients in the two groups had completed the 48-week follow-up. At week 48, 75.8% and 86.5% of the patients in the two groups, respectively, achieved undetectable plasma HBV DNA load (<128 copies/mL) (P=0.34). Compared with those achieving undetectable plasma HBV DNA load, patients failing to achieve viral suppression at week 48 had higher HBV DNA load (7.6 vs. 5.6 log10 copies/mL, P<0.001), and were more likely to be HBeAg-positive (86.7% vs. 23.2%, P<0.001) before starting tenofovir/lamivudine-containing cART. In multivariate analysis, only HBeAg positivity was associated with measurable plasma HBV DNA at week 48 (odds ratio, 11.36, 95% CI, 1.93-66.82, P=0.007).

Conclusions: In the treatment of lamivudine-resistant HBV infection in HIV-infected patients, add-on tenofovir achieved a similar rate of suppression of HBV replication to tenofovir/lamivudine-containing cART as the frontline regimen for HIV/HBV coinfection.

Keywords: HBV, HIV, lamivudine resistance

貳、本文

（一）前言

愛滋病毒(HIV)及 B 型肝炎病毒(HBV)感染途徑相同，均是藉由接觸患者血液及體液交換（如性行為、輸血以及母子間垂直感染）而傳染，因此 HIV 感染者也容易同時合併感染 HBV [1-3]。根據歐美地區 HBV 血清標記的流行病學調查，HIV 病患之慢性 HBV 感染（HBsAg 帶原大於六個月）盛行率約為 6-10%，相較於非 HIV 感染之一般人口 HBsAg 帶原率約為 0.5%-1%（歐美並非 HBV 高盛行區）高出許多 [4,5]；而台灣為 B 型肝炎感染之高盛行區，HIV 感染者之慢性 HBs Ag 帶原率與一般族群成年人的流行病學相似，約為 15%至 20% [2]。過去因 HIV 感染者存活時間較短，大多數患者在感染 HIV 後經過 6 至 8 年，就可能因發病（AIDS）而死於愛滋病毒感染相關的伺機性感染或腫瘤，因此無法觀察到慢性 HBV 感染相關的併發症發生。但是目前在高效能的抗 HIV 藥物的使用下，病患得以長期存活，因此慢性肝炎疾病被突顯出來，慢性 B 型肝炎感染相關的併發症已經成為歐美開發國家 HIV 病患需住院治療及導致死亡的主要原因 [6-8]。

合併感染 HIV 之慢性 HBV 患者，特別是已進展至 AIDS 發病狀態，相較於非 HIV 感染者之慢性 HBV 感染，前者血液中的 HBV 病毒量較高，且 HBeAg 較不容易消失。此外，HIV 與 HBV 合併感染的患者，其急性肝炎發作的頻率與 CD4 淋巴球數有關：CD4 淋巴球數越低，急性肝炎發作頻率就越高 [7]。Colin 等人 [6] 追蹤慢性 B 型肝炎（其中 HIV 感染者為 65 位）之男同性戀者，發現合併 HIV 感染者其血清肝功能轉氨酶指數（ALT）較無 HIV 感染者明顯偏高、血清 HBV DNA 病毒量亦較高，並且有較多機會發生肝硬化及肝癌。而其他的長期性觀察研究亦發現合併感染慢性 HBV 感染的 HIV 男同性戀患者，比非合併 HIV 感染的慢性 HBV 患者有更高發生肝硬化及肝癌的機會 [7-9]。

Lamivudine 為一種可以同時抑制 HBV 及 HIV 的核苷酸反轉錄酶的藥物，單獨 HIV 感染者服用 lamivudine 治療時使用的劑量每日為 300 mg，以相同劑量治療合併 HIV 與 HBV 感染者，治療一年之 HBeAg 陰轉率約為 22%至 29% [10,11]，HBV DNA <400 copies/mL(測不到)的比率約為 40%至 87%。然而治療合併感染 HIV 與 HBV 的患者時，常因為藥物副作用過劇或對 lamivudine 產生抗藥性病毒株，而需將 lamivudine 自高效能抗愛滋病毒治療處方中移除，此舉可能會讓在長期 lamivudine 抑制下的 HBV 急速活化，而導致肝功能的惡化 [12]。在 HIV 感染者中，lamivudine 發生 YMDD 之抗藥性突變的

機會，在治療 1 年時約為 25%，2 年約為 50%，4 年約為 90% [11,13]，且患者對 lamivudine 發生抗藥性突變時，HBV DNA 可能會再度升高而引起急性肝炎。因此，為了避免單一使用 lamivudine 可能造成的抗藥性發生，國外治療 HBV 與 HIV 感染之準則建議同時以二種抗 HBV 病毒藥物（如 adefovir 加 lamivudine，tenofovir 加 emtricitabine 等）來同時治療 HBV 與 HIV，以防止 HBV 發生突變 [14]。

過去有幾篇小型的臨床研究評估 tenofovir 對於合併 HBV 及 HIV 感染者的療效。在一研究 52 個 HBV/HIV 感染者比較 tenofovir 及 adefovir 之治療反應，HBV DNA 在使用 tenofovir 治療組下降 4.44 log，而使用 adefovir 治療組下降 3.21 log [15]；另一研究比較合併 tenofovir 及 lamivudine 治療與單獨使用 lamivudine 治療之差異，發現合併 tenofovir 及 lamivudine 治療一年可下降 HBV DNA 4.7log，而單獨使用 lamivudine 治療者下降 HBV DNA 3.0 log [16]。另外，對於 lamivudine 抗藥性病毒株，使用 tenofovir 併用 lamivudine 一年在 HBeAg 陽性者有 30%測不到 HBV DNA，而 HBeAg 陰性者有 82%測不到 HBV DNA，治療一年並未發現 tenofovir 抗藥性發生 [17]。

由於國內 HIV 及 HBV 盛行率較國外高出許多，對於治療後發生 lamivudine 抗藥性 HBV 病毒株時之後續處理十分棘手。台灣疾病管制署已通過可第一線使用新的抗 HBV 藥物 tenofovir 對合併 HIV 及 HBV 感染的患者做長期性的合併處方治療，以減少患者發生 HBV 抗藥性突變，改善患者預後 [18]。雖然目前台灣亦有其他的藥物(如 entecavir 及 adefovir)可做為 HBV 之治療，但此二種藥物無法同時治療 HIV 與 HBV 感染，需要與其他抗 HIV 核苷酸反轉錄酶抑制劑併用 [19,20]。並且在 HIV 感染者若單獨以此類藥物治療 HBV 可能引起 HIV 之交叉抗藥性(cross-resistance)，使日後抗 HIV 三合一療法之核苷酸反轉錄酶抑制劑（NRTI）組合治療失敗的機會增加[21,22]。長效型干擾素 peginterferon alfa-2a (Pegasys)雖然有效，且 IFN-α 不能治療 HIV，不會有 HBV 或 HIV 的抗藥性，但臨床使用證據仍不足 [23,24]。因此若患者需要同時治療 HIV 與 HBV 時，使用 tenofovir 加上加上 lamivudine 之合併療法可以減少 HBV 對 lamivudine 之抗藥性，但此建議目前並未有大型臨床研究證實。

本研究依據台灣疾病管制署目前對於 HBV 與 HIV 合併感染者治療之建議 [18]，對於 HIV 及 HBV 合併感染者在使用含有 lamivudine 高效能抗愛滋病毒藥物治療後發生 lamivudine 抗藥性時加上 tenofovir 之治療，以及第一線使用 tenofovir 治療之 HIV 及 HBV 合併感染者，在開始接受 tenofovir 治療後，監測追蹤患者之肝、腎功能、HBV DNA 之

變化、HBeAg 及 HBsAg 的陰轉率以及 HBV tenofovir 抗藥性之發生，以期了解在 HIV 感染者合併慢性 HBV 感染時，治療 HBV 之最佳藥物處方，並希望減少患者發生 HBV 抗藥性突變，改善患者預後，減少 HIV 感染者暴露於 B 型肝炎之感染危險。期望藉由本研究能了解本土 HIV 合併 HBV 感染病患之最佳治療處方，以提供臨床醫師做治療上之參考。

（二）材料與方法

研究地點：臺大醫院愛滋病研究中心

研究對象：在臺大醫院追蹤治療的 HIV 及 HBV 合併感染之病患；在臺灣其他愛滋專責醫院追蹤治療的 HIV 及 HBV 合併感染之病患。

研究期間：自 104 年 1 月 1 日迄 104 年 12 月 31 日。

研究方法

於研究期間預期完成之工作項目如下：

1. 延續過去研究，在104年度持續納入合併HIV與HBV感染、具有HBV lamivudine 抗藥性、並使用tenofovir 300 mg藥物治療之病患，以及自100年6月後使用tenofovir藥物作為第一線抗病毒藥物組合之HIV與HBV合併感染之病患。
2. 針對收集個案病患之基本資料，如性別、年齡、HIV感染危險因子、HIV感染時間、接受lamivudine治療之時間、CD4淋巴球之變化、血清HIV病毒量之變化、合併伺機性感染之有無、肝功能、肝炎、肝硬化等資料整理登錄。
3. 在病患進行tenofovir 300 mg藥物治療時，於第4、8、12、24、36、48週檢測HBV DNA之變化，每12週檢測肝功能及定量HBsAg之變化，於第48週檢測B型肝炎血清標記(HBeAg、anti-HBeAg)，評估是否可能發生HBV tenofovir之抗藥性，每24週檢測腎功能，於每次回診時記錄是否有tenofovir相關之藥物不良反應。藉由以上檢驗了解個案在tenofovir治療後對HBV感染控制之成效與藥物副作用之發生率。
4. 所有病患每12或24週檢測血液CD4淋巴球之變化、血清HIV病毒量之變化。
5. 在其他參與研究的愛滋專責醫院，納入自100年6月後使用tenofovir藥物作為第一線抗病毒藥物組合之HIV與HBV合併感染之病患，收集個案病患之基本資料，及

使用tenofovir治療期間之肝功能、肝炎、肝硬化等資料、CD4淋巴球之變化、血清HIV病毒量之變化、每24至48週HBV DNA之變化、B型肝炎血清標記(HBeAg、anti-HBeAg)等。

6. 若病患使用tenofovir治療48週以上，血中HBV DNA仍大於1000 copies/mL，則作DNA定序分析是否帶有tenofovir抗藥性相關之突變。
7. 收集以上臨床及病毒學相關資料，藉統計分析探討HIV及HBV合併感染者使用tenofovir治療之療效，與單獨使用lamivudine之對照組作比較，分析有無lamivudine抗藥性及其他變因是否會影響tenofovir治療之療效，及長期使用tenofovir治療之下，是否會造成tenofovir抗藥性突變之發生。

實驗研究操作方法

肝炎病毒血清標記之檢測

B 型肝炎病毒血清標記（HBs Ag、anti-HBs、HBe Ag、anti-HBc）之檢查利用 Commerical kits 以酵素免疫方法（Enzyme Immunoassay，ABBOTT Murex Anti-HBs、Murex Anti-HBc、Murex HBeAg/anti-HBe、Murex HBsAg）操作。

萃取 HBV 定量 HBV DNA 及 YMDD 基因定序

根據（Qiagen，Chatsworth，CA，USA）公司的操作手冊，利用 QIAamp 血液套組從 200uL 血清檢體中，萃取 HBV DNA 片段，並將 HBV 基因組位置 256 至 796 的核苷酸放大。聚合酶連鎖反應的產物以限制酶反應，之後將反應產物以 ethidium bromide 染色，以 3%洋菜膠跑電泳。利用此法，我們約能偵測到最低的 HBV DNA 敏感度（sensitivity）約為 100 copies/mL。經由全自動 ABI-DNA 定序機（Model 377A；Applied Biosystems，Foster City，CA，USA）的處理，證實聚合酶連鎖反應的產物基因包含有酥氨酸-甲硫氨酸-天門冬氨酸-天門冬氨酸變異株（tyrosine-methionine-asparate-asparate，YMDD mutant：

rtM204V，rtM204I 及 rtL180M）。第一對 HBV 的引子為 HBV

F1:5’-CCGATCCATACTGCGGAAC-3′（1261-1279） 及 HBV R1:

5’-GCAGAGGTGAAGCGAAGTGCA -3’（1600-1580）with anchor probe:

5′-TCTGTGCCTTCTCATCTGCCGGACC-3′（(1552-1576）and sensor probe:

5′-TCTTTACGCGGACTCCCC-3′（1533-1550）。第二對 HBV 的引子為 HBV F2:

5’-GCATGCGTGGAACCTTTGTG-3’（1232-1251）and HBV R2:

5’-CAGAGGTGAAGCGAAGTGC-3’（1599-1581）with anchor probe:

5′-CGGCGCTGAATCCCGCGGAC-3′（1436-1455）and sensor probe:

5′-ACGTCCTTTGTCTACGTCCCG-3′(1414-1434)。聚合酶連鎖反應的條件設定為：94℃30 秒，55℃1 分鐘，72℃2 分鐘共 30 回，及終回 72℃7 分鐘。第一回的 1- L aliquot 聚合酶連鎖反應產物，應用於第二回的聚合酶連鎖反應，反應條件與第一回相同。聚合酶連鎖反應產物大小期值約 416 bp，並以洋菜膠電泳確認。

（三）結果

截至 2015 年 10 月，針對具有 HBV lamivudine 抗藥性突變之 HIV/HBV 合併感染者共納入 33 位病患，平均追蹤 3.7 年；第一線使用 tenofovir 治療之 HIV/HBV 合併感染者則共納入 56 位病患，平均追蹤 2.4 年。兩組幾乎皆為男性 (100%及 98.2%)，帶有 lamivudine 抗藥性突變者平均年齡較大 (42±8 vs. 36±8 歲)，兩組病患 HBV 之基因型主要皆為 genotype B (84.8%及 83.9%)，且開始使用 tenofovir 治療前血中 HBV DNA 平均值 (6.1±2.1 vs. 6.0±2.2 copies/mL) 及 HBeAg 陽性率 (48.5% vs. 31.5%, *P* =0.17) 並無差異 (Table 1)。兩組各有 33 位及 52 位病患完成至少一年 (48 週) 的治療與追蹤，其中各有 75.8%及 86.5%的病患其血中 HBV DNA 已降至<128 copies/mL (測不到) (Figure 1&2)。在多變量分析中，惟有 HBeAg 陽性與經 48 週治療後血清仍可測得 HBV DNA 相關 (odds ratio, 11.36, 95% CI, 1.93-66.82, *P* =0.007) (Table 2)。兩組各有 1 位及 2 位病患在治療 48 週後血中 HBV DNA 仍 >1000 copies/mL，而同時間點 HIV viral load <40 copies/mL，目前未檢測到 tenofovir 相關之抗藥性突變。持續追蹤到治療後第二至五年，兩組病患血中 HBV DNA 曾達到測不到的比率分別為第二年：86.7% vs. 95%、第三年：92.6% vs. 96.6%、第四年及第五年：100% vs. 100% (Figure 2)。所有病患中只有 1 位病患在使用 tenofovir 36 週時發生血清肌酸酐上升而需停藥，其餘病患沒有發生任何嚴重副作用需要停用藥物。追蹤期間沒有任何病患發生 HBV 相關的急性肝炎。

（四）討論

研究結果顯示 tenofovir 對於帶有 lamivudine 抗藥性突變，及無 lamivudine 抗藥、第一線使用抗病毒藥物治療之 HIV/HBV 合併感染者，其治療效果一樣好，經 48 週治療後各有 75.8%及 86.5%的病患其血中 HBV DNA 可降至測不到的範圍 (<128 copies/mL)，而持續追蹤到治療後第二至五年，兩組病患血中 HBV DNA 曾達到測不到的比率分別為第二年：86.7% vs. 95%、第三年：92.6% vs. 96.6%、第四及第五年：100% vs. 100%。多變量分析中惟有 HBeAg 陽性與經 48 週治療後血清仍可測得 HBV DNA 相關，因此對於 HBeAg 陽性的 HIV/HBV 合併感染者，應長期追蹤 HBV 之治療效果。

在藥物的安全性方面，追蹤期間只有 1 位過去有糖尿病史的病人在使用 tenofovir 36 週時發生血清肌酸酐上升而需停藥，因此對於本身已有可能影響腎功能之慢性病患者(如糖尿病、高血壓等)，或併用其他可能有腎毒性的藥物時，應注意定期抽血追蹤腎功能。

目前兩組病患平均追蹤 3.7 及 2.4 年治療的結果，顯示 tenofovir 的治療效果佳，且沒有明顯副作用。然而其他 HBV 的治療藥物多有因長期使用而發生 HBV 抗藥性突變的問題 (lamivudine 最易發生抗藥性突變，其他藥物如 entecavir、adefovir 等雖較少見但仍有病例報告)，因此未來也需要繼續追蹤長期使用 tenofovir 治療是否可能產生 HBV 抗藥性突變的問題，尤其是少數幾位服藥順從性不佳導致血中 HIV 及 HBV 病毒量增高的病患，是否會類似 HIV 一樣因藥物濃度不穩定而增加 HBV 抗藥性突變的發生。

（五）結論與建議

對於 HIV/HBV 合併感染者，無論是曾接受 lamivudine 治療並帶有抗藥性突變的感染者，或是第一線使用抗病毒藥物的病患，都應該依照目前的治療指引選用含有 tenofovir 的抗愛滋病毒藥物組合，以有效抑制 HBV 病毒複製，避免發生慢性 B 型肝炎之相關併發症。未來則需注意追蹤長期使用藥物之可能副作用，及是否可能和其他抗 HBV 藥物一樣發生 HBV 抗藥性突變。

對政策之具體建議:對於 HIV/HBV 合併感染者為避免發生慢性 B 型肝炎之相關併發症應選用含有 tenofovir 的抗愛滋病毒藥物組合。

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參、圖表

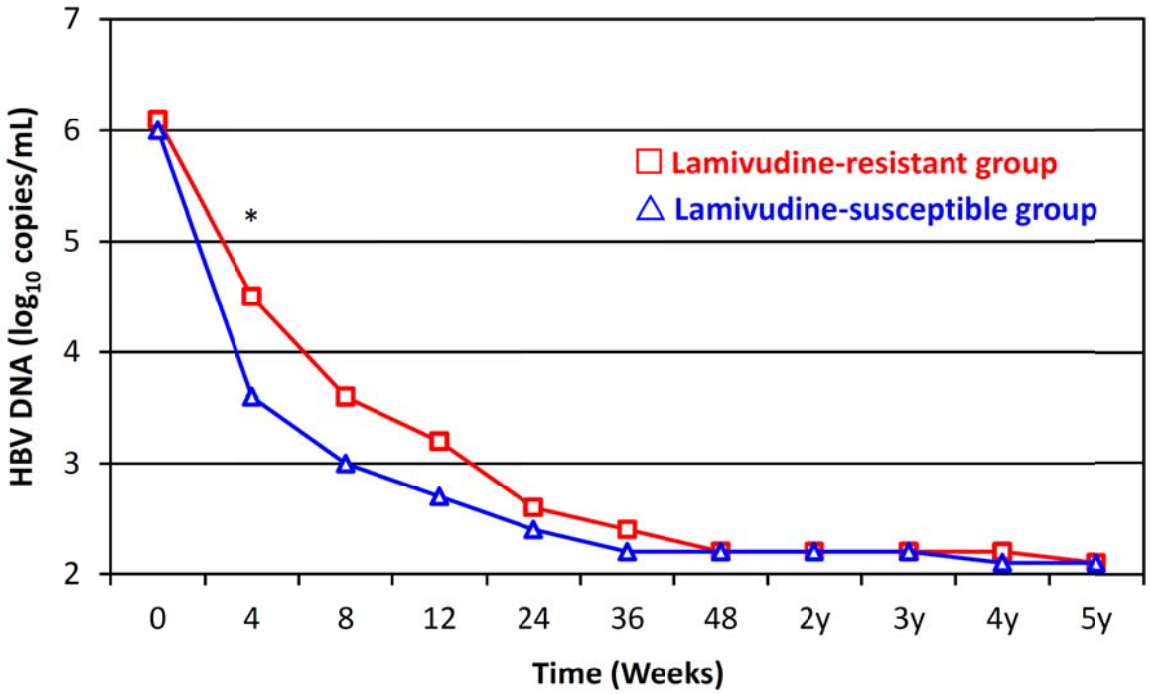
Table 1. Baseline characteristics of HIV-infected patients with LAM-resistant and -susceptible HBV

	LAM-resistant N=33	**LAM-susceptible N=56**	***P* value**
Age, years	42 ± 8	36 ± 8	**<0.001**
Male sex	33 (100)	55 (98.2)	0.99
HBV genotype B	28 (84.8)	26 (26/31, 83.9)	0.99
Previous LAM use, years	6.3 ± 3.5	NA	
Duration of LAM use to LAM resistance, years	3.4 ± 3.2	NA	
HBV DNA at baseline, log10 copies/mL	6.1 ± 2.1	6.0 ± 2.2	0.83
HBV DNA at baseline> 5 log10 copies/mL	19 (57.6)	33 (58.9)	0.92
HBeAg positivity at baseline	16 (48.5)	17 (17/54, 31.5)	0.17
AST or ALT >2X ULN at baseline	4 (12.1)	11 (19.6)	0.53
Hepatitis flares in prior one year	6 (18.2)	NA	
Parenchymal liver disease or cirrhosis at baseline	10 (10/31, 32.2)	10 (10/39, 25.6)	0.73
Chronic HCV infection at baseline	0	2 (3.6)	0.79
CD4 count at baseline, cells/μL	552 ± 376	249 ± 218	**<0.001**
HIV RNA level <200 copies/mL	31 (93.9)	NA	
Diabetes mellitus	1 (3.0)	0	0.94
Undetectable HBV DNA (<128 copies/mL) at week 48	25 (75.8)	45 (86.5)	0.34

Table 2. Univariate and multivariate logistic regression analyses of variables associated with measurable HBV DNA at week 48 (8 and 7 patients in LAM-R and LAM-S groups, respectively)

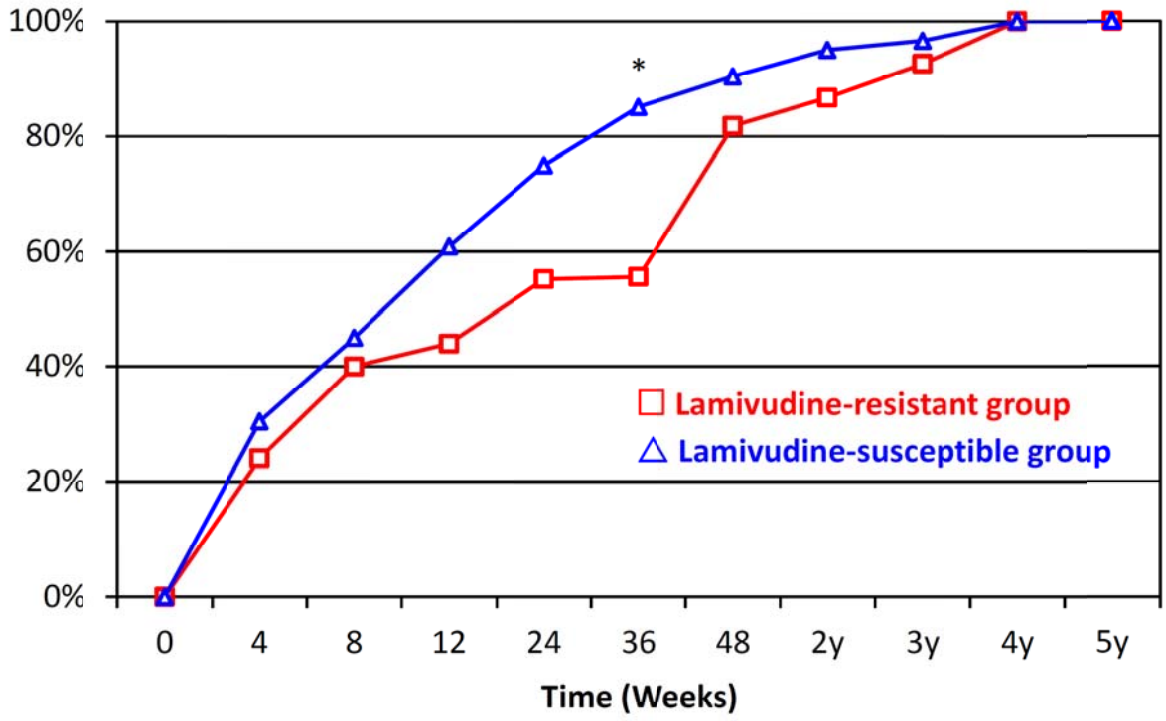
Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Baseline HBV DNA, log10 copies/mL	1.57 (1.15-2.14)	**0.004**	1.19 (0.82-1.73)	0.37
HBeAg positivity	17.11 (3.53-83.02)	**<0.001**	11.36 (1.93-66.82)	**0.007**
Age, years	0.95 (0.88-1.02)	0.16		
Lamivudine resistance	2.06 (0.67-6.34)	0.21		
HBV genotype B	2.57 (0.30-22.37)	0.39		
CD4 count at baseline, cells/μL	1.00 (0.998-1.002)	0.87		
Baseline HIV RNA, log 10 copies/mL	0.79 (0.56-1.12)	0.18		

Figure 1. Changes of HBV DNA on tenofovir treatment in the two groups



LAM-R	33 (33)	33 (25)	33 (25)	33 (25)	33 (29)	33 (27)	33 (33)	30 (30)	27 (27)	23 (23)	10 (10)
LAM-S	56 (56)	54 (46)	54 (20)	54 (46)	53 (48)	53 (47)	52 (52)	40 (40)	29 (29)	10 (10)	4 (4)

Figure 2. Percentage of patients with undetectable HBV DNA (<128 copies/mL) in the two groups



LAM-R	33 (33)	33 (25)	33 (25)	33 (25)	33 (29)	33 (27)	33 (33)	30 (30)	27 (27)	23 (23)	10 (10)
LAM-S	56 (56)	54 (46)	54 (20)	54 (46)	53 (48)	53 (47)	52 (52)	40 (40)	29 (29)	10 (10)	4 (4)

附件九

計畫編號：MOHW104-CDC-C-114-000301

衛生福利部疾病管制署 104 年委託科技研究計畫

計畫名稱：愛滋病毒感染者接種七價結合型肺炎疫苗五年後血清抗體變化

年 度 研 究 報 告

執行機構：國立台灣大學醫學院附設醫院

計畫主持人：洪健清

研究人員：洪健清、張淑媛、鄭琬鸊、劉玟君、蘇意青

執行期間：104 年 1 月 1 日至 104 年 12 月 31 日

＊本研究報告僅供參考，不代表本署意見，如對媒體發布研究成果應事先徵求本署同意*

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壹、中文摘要

接種七價結合型肺炎鏈球菌疫苗已經在非洲被證實可以降低愛滋病毒感染者發生復發性侵犯性鏈球菌感染。美國成人疫苗接種委員會已經在 2012 年正式將結合型疫苗接種建議為十九歲以上的愛滋病毒感染的成人可以接種的疫苗。不過，愛滋病毒感染的成人接受了結合型肺炎疫苗後的長期血清抗體的變化相關研究仍然很少。我等在 2008-2010 年之間針對四百多位愛滋病毒感染的成人，提供免費七價結合型肺炎疫苗接種，初步 24 與 48 週的追蹤結果已經發表於 Vaccine 2012 (May)。到了 2014 年，受試者接種疫苗逐漸屆滿五年。我們針對 115 對接種一劑或兩劑的受試者，定量五年以來的血清抗體效價，以評估是否有追加接種的必要性，並且評估和抗體消失或持續存在的相關因素。我們發現，接種二劑的蛋白結合型肺炎鏈球菌疫苗受試者，相較於接受一劑的受試者，在五年以後，還能夠對至少兩種或兩種以上的肺炎鏈球菌莢膜型產生明顯血清抗體反應(adjusted odds ratio [AOR] 1.71, 95% confidence interval [CI] 1.10-2.65, p=0.016)。結果已經投稿於 Journal of International AIDS Society (Aug 2015)。

關鍵詞：肺炎鏈球菌疫苗、肺炎鏈球菌、肺炎、侵犯性肺炎鏈球菌感染、愛滋病毒感染、高效能抗病毒組合治療

貳、本文

（一）前言

相較於非愛滋病毒感染者，愛滋病毒感染者有較高的風險發生侵犯性的肺炎鏈球菌的感染 (1-5).在組合式的抗愛滋病毒藥物治療出現之前，愛滋病毒感染者發生侵犯性的肺炎鏈球菌感染的風險高到百倍之多(6); 即便在有組合式的抗愛滋病毒藥物治療時代，愛滋病毒感染者發生侵犯性的肺炎鏈球菌感染的風險依然高到 35-60 倍之多(4, 7, 8)。

為了預防肺炎鏈球菌所造成的疾病，美國衛生部建議愛滋病毒感染者接種 23 價多醣體肺炎疫苗(9). 不過，愛滋病毒感染者接種多醣體的肺炎鏈球菌疫苗後所產生的抗體反應並不好，而且不能持久(10-12)；再者，目前並沒有任何隨機分組的臨床試驗證實接種 23 價多醣體肺炎疫苗可以降低愛滋病毒感染者發生肺炎鏈球菌相關的肺炎(13).

在 2000 上市的七價蛋白結合型肺炎鏈球菌疫苗可以誘發 T 細胞相關的免疫反應，因此具有誘發產生記憶細胞重新接觸到相關抗原時，這些劑疫細胞可以立即產生抗體(14, 15) 。 過去多年來以經有不少的臨床研究證實愛滋病毒感染者接種七價蛋白結合型肺炎鏈球菌疫苗後，可以產生較好的抗體反應(15-18). 近年的臨床試驗也證實，相較於安慰劑，接種七價蛋白結合型肺炎鏈球菌疫苗可提供 74%的保護效果，降低再發侵犯性肺炎鏈球菌的風險 (19). 不過，相關愛滋病毒感染者接種七價蛋白結合型肺炎鏈球菌疫苗併糗，並且接受長期追蹤的研究並不多見。因此，在本研究中，我們即是想評估接種七價蛋白結合型肺炎鏈球菌疫苗五年後的血清抗體變化；同時，我們也想針對過去已經接受 23 價疫苗的受試者比較其抗體血清效價的變化。

（二）材料與方法

研究方法

1. 受試者：

年滿 20 歲的愛滋病毒感染者只要未曾接種過肺炎鏈球菌疫苗，在 2008 年 10 月到 2010 年 6 月期間，接種了七價蛋白結合型疫苗。排除條件是: 懷孕、過去六個月中使用免疫抑制劑或免疫球蛋白、或接受化學治療。本研究業經台大醫院倫委會同意通過後執行，受試者必須填寫受試者同意書後方可以參加試驗。受試者接種疫苗後，依然接受例行的愛滋病毒藥物治療或追蹤。

2. 疫苗施打：

病患在五年前加入研究時，都陸續接種一劑或者是兩劑的七價蛋白結合型肺炎鏈球菌疫苗。疫苗接種後，每一位受試者都持續接受門診定期追蹤。從定期收取的血液檢體，我們會檢測針對四種肺炎鏈球菌血清型的抗體反應，一直持續五年之久。

3. 實驗室檢驗：

血漿愛滋病毒量和 CD4 定量都是使用常規醫院實驗室檢工具。.

針對四種肺炎鏈球菌血清型的抗體檢測，則式在台大醫學院醫事技術學系張淑媛老師實驗室中進行。所用的檢測方式是依照國際文獻所發表的 ELISA 方式，略做修式的檢測方式(21)。這個方法也是過去我們關於肺炎疫苗接種研究的論文中所用的方法。

4. 統計分析：

我們會使用統計軟體進行所有統計分析。因為血清中抗體量陸續的檢測會著時間前後點相關，因此我們採取 generalized estimating equations (GEE) 來比較和校正相關影響抗體變化的因素(25)。

（三）結果

截至 2015 年 10 月為止，一共有 221 位預期觀察期間距離接種蛋白結合型肺炎鏈球菌疫苗後，已經達到五年的追蹤時間。其中 109 接種一劑，112 接種二劑。但是在一劑組中，有 7 位病患因為死亡(一位)，或轉介其他醫院追蹤、失去行蹤等，並未全程完成五年的血清追蹤。在二劑組中，也有 9 人轉介其他醫院追蹤或者失去行蹤。

表一顯示兩組的臨床基本資料和疫苗接種五年後的 CD4 和病毒量的變化。兩組是經過配對的受試者，因此基礎臨床資料相似，包括年紀、性別、感染愛滋病毒的險行為、CD4 和病毒量。兩組各有 71-72%的比例在接種疫苗時已經在服用抗愛滋病毒組合藥物治療。而在第五年的追蹤時，已經都有超過 92%受試者病毒藥物治療。

表二顯示出兩組受試者在第五年依然能夠檢測到對四種肺炎鏈球菌莢膜型中產生二倍或以上的血清抗體濃度的反應。

表三顯示出對於其中任一種、任二種、任三種或四種肺炎鏈球菌莢膜型中產生二倍

或以上的血清抗體濃度的反應的結果。

表四則是分析能夠對至少兩種或兩種以上的肺炎鏈球菌莢膜型產生明顯血清抗體反應的相關因子。使用 GEE 的統計分析方式，我們發現接種二劑的蛋白結合型肺炎鏈球菌疫苗受試者，相較於接受一劑的受試者，在五年以後，還能夠對至少兩種或兩種以上的肺炎鏈球菌莢膜型產生明顯血清抗體反應(AOR 1.71, 95% CI 1.10-2.65, p=0.016)。

（四）討論

蛋白結合型肺炎鏈球菌疫苗已經正式被建議使用於免疫功能損的病患，含愛滋病毒感染者。但是，免疫系統缺損的病患，應該接種的適當劑數，是否要追加接種等等，目前仍不清楚。國際間的研究大多著重於短期的抗體血清研究。我們的研究是少數追蹤較大族群的愛滋病毒感染者，並且大多數開始接種時或者在追蹤當中接受抗病毒藥物治療，維持較低的病毒量和較好的免疫功能，同時，也是極為少數持續追蹤五年的血清抗體反應的長期追蹤研究。我們定義的產生明顯的血清抗體反應，和過去我們一系列的研究一樣，同時追蹤的肺炎鏈球菌夾膜型的血清型，也是一樣，因此我們未來有機會同時比較接種多醣體疫苗和蛋白結合型疫苗五年追蹤的抗體變化。這些資料將可提供國內或國際間對於愛滋病毒感染者的肺炎疫苗接種建議做為參考。

此研究中，我們發現接種二劑的蛋白結合肺炎鏈球菌疫苗，較於接受一劑的受試者，在多變項洗分析中，在五年依然有較高的機會(79%)維持明顯的血清抗體反應。因此，純粹就血清抗體的量做為研究觀察重點，我們的研究支持愛滋病毒感染者接種兩劑蛋白結合肺炎鏈球菌疫苗。

（五）結論

對政策之具體建議:對於 HIV 感染者若要接種蛋白結合肺炎鏈球菌疫苗應接種兩劑效果較佳。

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參、圖表

Table 1. Characteristics of patients receiving one versus two doses of PCV-7

	1-dose (n = 109)	2-dose (n = 112)	p
Age, mean (SD), years	35.8 (10.1)	36.1 (10.8)	0.789
Male (%)	104 (95.4)	108 (96.4)	0.746
Risk group (%)			0.520
Homosexual/bisexual male	93 (86.1)	87 (80.6)	
Heterosexual	13 (12.0)	17 (15.7)	
Injecting drug user	2 (1.9)	4 (3.7)	
Treatment status (%)			
On cART at baseline	77 (70.6)	81 (72.3)	0.782
On cART at end of 5-year	94 (92.2)	95 (92.2)	0.984
CD4 lymphocyte count, cells/μL, median (IQR)			
Nadir CD4	240 (79-387)	229 (51-450)	0.737
Baseline CD4	407 (244-583)	446 (252-591)	0.543
Baseline CD4 <200 (%)	13 (11.9)	15 (13.4)	0.743
Baseline CD4 200-349 (%)	25 (22.9)	26 (23.2)	0.961
Baseline CD4 350-499 (%)	31 (28.4)	26 (23.2)	0.375
Baseline CD4 ≥500 (%)	40 (36.7)	45 (40.2)	0.595
End of 5-year CD4	550 (426-735)	592 (433-749)	0.476
End CD4 <200 (%)	6 (5.6)	11 (10.0)	0.229
End CD4 200-349 (%)	21 (19.6)	14 (12.7)	0.167
End CD4 350-499 (%)	32 (29.9)	26 (23.6)	0.297
End CD4 ≥500 (%)	46 (43.0)	59 (53.6)	0.117
Plasma HIV RNA (PVL), $\log_{10}$ copies/ml			
Peak PVL, median (IQR)	4.9 (4.3-5.4)	5.8 (4.4-5.6)	0.025
Baseline PVL, median (IQR)	2.2 (1.6-4.0)	1.7 (1.6-3.9)	0.314
End of five year PVL, median (IQR)	1.3 (1.3-1.3)	1.3 (1.3-1.3)	0.974
Undetectable PVL at baseline (%)	48 (44.0)	52 (46.4)	0.721
Undetectable PVL at end of 5yr (%)	84 (82.4)	84 (81.6)	0.882
Co-morbidities pre-vaccination (%)			
Chronic HBV co-infection	20 (19.0)	20 (17.9)	0.821
Chronic HCV co-infection	6 (5.6)	5 (4.5%)	0.710
Isolated anti-HBc	48 (47.5)	45 (42.5)	0.463
Chronic pulmonary disease	1 (0.9)	6 (5.4)	0.119
Congestive heart failure	1 (0.9)	3 (2.7)	0.622
Diabetes mellitus	4 (3.7)	2 (1.8)	0.441

Table 2. Percentage of HIV-infected adults with persistent immune responses defined by a 2-fold or more IgG rise to at least two serotypes in the 2nd, 3rd, 4th and 5th years following one or two doses of PCV7 by intention-to-treat (ITT) and per-protocol (PP) analyses (primary endpoint).

	1-dose	2-dose	*P* value*
ITT			
Year 2	67.9	78.6	0.072
Year 3	64.2	71.4	0.251
Year 4	66.1	71.4	0.388
Year 5	57.8	69.6	0.067
PP			
Year 2	67.4	78.2	0.079
Year 3	61.7	73.3	0.084
Year 4	66.3	76.3	0.128
Year 5	61.8	76.0	0.026

Table 3. Sequential geometric mean titers (95% confidence interval) of specific anti-capsular immunoglobulin (IgG) antibodies to S. pneumoniae serotypes 6B, 14, 19F, and 23F in the 2nd to 5th years following vaccination with one or two doses of PCV7 (secondary endpoint).

	1-dose	2-dose	*P* value
Anti-6B serotype IgG levels, μg/ml			
Baseline	0.855 (0.743-0.985)	0.774 (0.643-0.843)	0.131
Year 2	2.023 (1.780-2.300)	1.943 (1.691-2.231)	0.670
Year 3	1.896 (2.149-1.747)	1.747 (1.537-1.987)	0.367
Year 4	1.948 (1.714-2.214)	1.760 (1.544-2.006)	0.272
Year 5	1.790 (1.571-2.040)	1.734 (1.519-1.979)	0.734
Anti-14 serotype IgG levels, μg/ml			
Baseline	1.992 (1.656-2.397)	1.905 (1.574-2.308)	0.739
Year 2	8.493 (7.037-10.253)	9.816 (8.182-11.776)	0.274
Year 3	7.706 (6.359-9.337)	8.696 (7.725-10.439)	0.366
Year 4	7.704 (6.355-9.338)	8.803 (7.345-10.549)	0.317
Year 5	6.980 (5.704-8.543)	8.216 (6.760-9.985)	0.250
Anti-19F serotype IgG levels, μg/ml			
Baseline	1.917 (1.648-2.230)	1.608 (1.354-1.910)	0.130
Year 2	3.254 (2.847-3.719)	3.265 (2.855-3.734)	0.973
Year 3	3.021 (2.649-3.446)	2.986 (2.633-3.387)	0.899
Year 4	3.277 (2.862-3.753)	3.020 (2.646-3.446)	0.392
Year 5	3.049 (2.682-3.477)	2.892 (2.537-3.295)	0.566
Anti-23F serotype IgG levels, μg/ml			
Baseline	0.734 (0.643-0.843)	0.644 (0.548-0.757)	0.020
Year 2	1.840 (1.554-2.180)	1.862 (1.574-2.202)	0.924
Year 3	1.709 (1.454-2.009)	1.624 (1.385-1.904)	0.654
Year 4	1.711 (1.444-2.028)	1.567 (1.343-1.829)	0.448
Year 5	1.555 (1.315-1.839)	1.502 (1.287-1.752)	0.760

Table 4. Factors associated with significant antibody responses to at least 2 serotypes

	2nd year				3rd year				4th year				5th year			
	AOR	**95% CI**		***p***	**AOR**	**95% CI**		***p***	**AOR**	**95% CI**		***p***	**AOR**	**95% CI**		***p***
Age (continuous)	0.986	0.962	1.011	0.259	0.981	0.957	1.006	0.133	0.984	0.961	1.008	0.190	0.984	0.960	1.008	0.195
Two doses vs. one dose	**1.786**	**1.123**	**2.840**	**0.014**	**1.623**	**1.027**	**2.566**	**0.038**	**1.604**	**1.024**	**2.513**	**0.039**	**1.711**	**1.104**	**2.652**	**0.016**
On cART vs. untreated	1.260	0.621	2.556	0.522	1.763	0.897	3.466	0.100	**1.793**	**0.950**	**3.384**	**0.071**	**2.156**	**1.162**	**4.000**	**0.015**
HBsAG-positive	1.001	0.510	1.965	0.999	1.031	0.528	2.017	0.928	0.896	0.469	1.714	0.741	0.860	0.459	1.611	0.637
Anti-HCV-positive	1.368	0.535	3.497	0.513	1.729	0.647	4.616	0.275	1.602	0.703	3.650	0.262	1.270	0.566	2.849	0.563
Baseline PVL >10^5 copies/ml	1.909	0.628	5.809	0.255	2.196	0.808	5.970	0.123	1.718	0.673	4.382	0.258	1.713	0.643	4.560	0.281
Time-updated PVL <20 copies/ml	1.022	0.492	2.125	0.954	0.939	0.491	1.796	0.848	0.802	0.421	1.530	0.504	0.766	0.363	1.618	0.485
Nadir CD4 count <200 cells/μL	1.251	0.691	2.266	0.459	1.205	0.669	2.171	0.534	1.268	0.716	2.246	0.416	1.424	0.805	2.518	0.225
Time-updated CD4 counts	1.084	0.937	1.253	0.277	0.993	0.880	1.120	0.908	1.105	0.986	1.239	0.085	**1.131**	**1.012**	**1.265**	**0.031**

附件十

計畫編號：MOHW104-CDC-C-114-000301

衛生福利部疾病管制署 104 年委託科技研究計畫

愛滋病毒感染者的梅毒發生率及相關因子探討

年 度 研 究 報 告

執行機構：台大醫院

計畫主持人：林綺英

研究人員：劉玟君

執行期間：104 年1 月1 日至104 年12 月31 日

＊本研究報告僅供參考，不代表本署意見，如對媒體發布研究成果應事先徵求本署同意*

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中文摘要

梅毒是愛滋病毒感染者常見的性病之一, 兩者間有相互危害的交互作用,梅毒會促進愛滋病毒的傳染,而感染梅毒會增高病毒複製和降低CD4數值。同時許多流行病學研究也都顯示愛滋病毒感染者發生梅毒感染後容易發生C型肝炎的急性感染。因此偵測梅毒的發生率是評估愛滋病毒感染者在公衛上防治效果的一個策略指標。 在本研究中我們採用兩個世代追蹤的愛滋病毒感染者規則在台大醫院接受愛滋醫療照護下發生梅毒的發生率. 第一個族群是在西元2009-2014年間, 凡是在台大醫院就醫而且有接受梅毒血清檢測至少一次以上的病患,都是我們回溯性查閱病歷資料的對象。自2009年到2014年止，共有3,102位愛滋病毒感染者在臺大醫院追蹤及治療愛滋病毒感染,總觀察人年為11019.1，梅毒發生率為10.95每100人年(95% CI, 10.34-11.57)；其中，2009年至2010年止，梅毒發生率為7.82每100人年(95% CI, 6.73-8.91)，但在2011年至2012年止，梅毒發生率增加至11.54每100人年(95% CI, 10.45-12.63)，2013年至2014年止，梅毒發生率更是高達14.70每100人年(95% CI, 13.59-15.80)。其次,我們又針對在2012年到2015年間在台大醫院開始接受抗愛滋病毒藥物的病患,其中扣除15位個案未服用HAART滿30天，並排除59位個案開始服用HAART超過30天、卻未在研究期間追蹤RPR價數，將剩餘796位開始服用HAART超過30天、有固定追蹤RPR價數的個案納入進一步統計分析。分析結果指出，198位（24.9%）個案發生梅毒感染，梅毒發生率為24.87%；自2012年7月1日至2015年10月31日止，總觀察人年為1190.4，梅毒發生率為16.63每100人年(95% CI, 14.32-18.95)。我們發現, 與沒有梅毒感染之個案相比，開始接受HAART治療後，有梅毒感染的個案較年輕（平均年齡30.7歲vs. 33.1歲），大多是男男間性行為者(98.5% vs. 92.6%)。與沒有梅毒感染的個案相比，有梅毒感染者在接受HAART治療前的CD4顆數及病毒量較高，但未達統計上顯著意義。有梅毒感染的個案當中，開始接受HAART治療後，第一次梅毒感染的平均年齡為31.8歲；開始接受HAART治療後，直到第一次梅毒感染的平均天數為385.5天；開始接受HAART治療後，90天內發生梅毒感染則有17位（8.6%）。

關鍵詞：梅毒，性病，愛滋病毒感染，抗愛滋病毒藥物，發生率

英文摘要

Syphilis and HIV infection are mutually detrimental in that syphilis increases HIV replication and decreases CD4 counts, while HIV infection accelerate the progression of syphilis, from infection to development of complications. In this study, we used two cohorts in two different study periods to estimate the trends of syphilis in HIV-positive patients. Between 2009 and 2014, 3102 HIV-positive patients who had been followed at the NTUH with more than 1 RPR test were included. We found that the incidence rate of syphilis increased from 7.82 per 100 person- (95% CI, 6.73-8.91) in 2009-2010，to 11.54 per 100 person-years 95% CI, 10.45-12.63) in 2011-2012, to 14.70 per 100 person-years (95% CI, 13.59-15.80) in 2013-2014. In the second cohort of patients who sought HIV care at the NTUH between June 2012 and July 2015, 870 patients started combination antiretroviral therapy. We included 796 patients who continued antiretroviral therapy for 30 days or longer and had follow-up of RPR for statistical analysis。We found that 198 patients（24.9%）developed one or more episode of syphilis; with a total observation duration of 1190.4 person-years, The incidence rate of syphilis was 16.63 per 100 person-years(95% CI, 14.32-18.95). Compared with those without syphilis in the second cohort, patients with syphilis were younger (mean age, 30.7 vs. 33.1 years）and were more likely to be MSM. Themena interval from initiation of combination antiretroviral therapy to the first episode of syphilis was 385.5 days; 17 patients (8.6%) had the first episode of syphilis with 90 days of initiation of antiretroviral therapy within 90 days of cART。

Keywords: Syphilis, HIV infection, MSM, cART, HAART

一、前言

自1996年開始，高效能抗反轉錄病毒療法(highly active antiretroviral therapy，以下簡稱HAART)廣泛運用在愛滋病的治療，愛滋病的致死率大幅下降、感染者存活時間延長，不能治癒的愛滋病已經成為慢性病。HAART改善了病患的免疫功能、有效控制病毒的複製，病患在規律服藥之下，大多能維持正常的生活作息。根據疾病管制署的統計，截至2014年12月止，國人感染通報個案為28711人，新近感染愛滋病毒的危險因子，主要還是以同性間性行為為主要傳染途徑。2009年愛滋病毒感染通報人數總共有18378人，其中，有21%同時被診斷出愛滋病毒和梅毒感染；從通報時間更可觀察到，有8.2%已經是愛滋病毒感染者在後來追蹤中被通報有梅毒感染[1]。顯而易見，這些愛滋病毒感染者其性行為仍然活躍。

在臨床上我們也發現，已經診斷是愛滋病毒感染者、又再次感染性病的情形有逐漸增加的趨勢。根據李欣純等人於2005年在門診收集的資料顯示，123位愛滋病毒感染者，有8.9%在收案的同時被診斷感染性病，梅毒是最常見的性病感染[2]；同一研究團隊在另一個研究中也發現，117位進入個案管理計畫追蹤一年的愛滋病毒感染者，有10.4%的個案有新發生的梅毒感染，這些個案皆為男男間性行為者（men who have sex with men，以下簡稱MSM）；CD4數值越高及娛樂性藥物的使用與新發生的梅毒感染有顯著相關[3]。陳嬿今等人使用健保資料庫，分析2000年至2010年，台灣愛滋病毒感染者性病感染的發生率，研究結果發現，梅毒感染是愛滋病毒感染者最常見的性病診斷，梅毒發生率為3.82每100人年(95% CI, 3.68-3.96)[4]。

愛滋病患者其性病感染率高，這意謂性活躍之愛滋病毒感染者並未採取安全性行為來保護自己。根據國內的研究顯示，MSM之愛滋病毒感染者比起異性戀或靜脈藥癮者，更容易會從事危險性行為[3]；另一研究也指出，愛滋病毒感染者比起非感染者，更有可能從事無保護性的肛交行為[5]。MSM之愛滋病毒感染者若從事無保護措施之肛交行為，無可避免地會造成愛滋病毒感染和性病的傳播持續進行[6]。隨著近年性病疫情爆發的同時，也發現在MSM族群中，意圖與不固定性伴侶從事不安全的肛交性行為(barebacking)有增加的趨勢[7]。舊金山的研究指出，MSM之愛滋病毒感染者是重複梅毒感染的高危險群（OR, 4.7; 95%CI, 1.8-12.0）[8]。愛滋病毒感染者有新的梅毒感染，與危險性行為的復發、年輕MSM者、從網路上尋找性伴侶有相關性[3, 9]。另外，台灣近期的研究也指出，正在接受HAART治療的MSM愛滋病毒感染者，其梅毒發生率比起尚未接受

HAART治療的個案還要來的高（8.3每100人年 vs. 19.3每100人年）[10]。而台大醫院在橫斷式研究中則發現，規則服用HAART之MSM愛滋病毒感染者，CD4數值越高、與同是HIV感染者發生性行為未採取安全措施（serosorting）及娛樂性藥物的使用，與梅毒感染有相關性[11]。台灣MSM之愛滋病毒感染者新診斷C型肝炎感染的發生率有增加趨勢，而新診斷C型肝炎感染與梅毒感染有顯著相關[12]。C型肝炎感染之治療，較其他性病相比，更是複雜及困難，無疑是防疫的另外一大困境及挑戰。

由於台灣還是以同性間性行為為主要愛滋病毒傳染途徑，我們很擔憂性行為活躍之MSM愛滋病毒感染者從事危險性行為，除了會暴露在性病感染的風險之外，也會繼續將愛滋病毒傳染給非感染者，造成愛滋病防治上的一大阻礙。期望本研究能提供目前愛滋病毒感染者梅毒感染盛行率及發生率概況，以提供相關單位參考。

二、材料與方法

研究對象是在2009-2014年間在台大就醫而且有接受梅毒血清抗體檢測的愛滋病毒感染病患。同時, 我們也在找尋在2012-2015年間在台大醫院開始接受抗愛滋病毒藥物的病患進行相同的研究。後面的族群有95%加入個管計畫,我們有機會可以評估行為和感染梅毒間的關係。

研究進行的方法，是由研究主持人和研究助理，定期每三個月回顧病歷記載，將病患的梅毒檢查及梅毒相關治療、定期CD4和血漿病毒量、病程中出現B型和C型肝炎感染及其使用抗病毒藥物組合、藥物副作用，採用制式的Excel紀錄。研究主持人和研究助理將整理出2009-2015年的資料，並分析這些紀錄資料，整理供院內照護愛滋病毒感染者的醫療同仁參考，以改善我們的愛滋照護水準。

三、結果

自2009年到2014年止,共有3,102位愛滋病毒感染者在臺大醫院追蹤及治療愛滋病毒感染,並在研究期間有追蹤梅毒血清學檢查(Rapid Plasma Reagin titer, RPR titer)；其中，有2,798位（90.2%）感染者之RPR檢測次數為兩次或以上，將其納入本研究發生率之計算與分析。在這2,798位愛滋病毒感染者中，有820位（29.3%）於研究期間有過一次或一次以上之梅毒感染 (圖一),與沒有梅毒感染之個案相比,有梅毒感染的個案較年輕(平均年齡32.5歲vs. 36.7歲）、大多是男男間性行為者(87.3% vs. 69.9%)、B肝表面抗體較多

人呈現陽性（56.2% vs. 48.6%），而且梅毒血清效價在收案開始就呈現四倍或四倍以上者也比沒有梅毒感染者多（39.3% vs. 13.3%）。而有梅毒感染個案與沒有梅毒感染相比，兩組在B型肝炎表面抗原（13.7% vs. 14.8%）與C型肝炎抗體（7.3% vs. 9.3%）的盛行率，在統計學上均沒有明顯差異。在820位有梅毒感染之個案中，第一次發生梅毒感染後，有68位（79.1%）個案發生C型肝炎感染。

自2009年至2014年為止，總觀察人年為11019.1，梅毒發生率為10.95每100人年(95% CI, 10.34-11.57)；其中，2009年至2010年止，梅毒發生率為7.82每100人年(95% CI, 6.73-8.91)，但在2011年至2012年止，梅毒發生率增加至11.54每100人年(95% CI, 10.45-12.63)，2013年至2014年止，梅毒發生率更是高達14.70每100人年(95% CI, 13.59-15.80) (圖二)。

自2012年6月至2015年7月，在台大醫院就診之愛滋病毒感染者，有870位開始使用HAART (圖三) 。自2012年7月1日至2015年10月31日，我們排除15位個案未服用HAART滿30天，並排除59位個案開始服用HAART超過30天、卻未在研究期間追蹤RPR價數，將剩餘796位開始服用HAART超過30天、有固定追蹤RPR價數的個案納入進一步統計分析。分析結果指出，198位（24.9%）個案曾有過梅毒感染，梅毒盛行率為24.87%；自2012年7月1日至2015年10月31日止，總觀察人年為1190.4，梅毒發生率為16.63每100人年(95% CI, 14.32-18.95)。

與沒有梅毒感染之個案相比，開始接受HAART治療後，有梅毒感染的個案較年輕（平均年齡30.7歲vs. 33.1歲），大多是男男間性行為者(98.5% vs. 92.6%)。與沒有梅毒感染的個案相比，有梅毒感染者在接受HAART治療前的CD4顆數及病毒量較高，但未達統計上顯著意義。有梅毒感染的個案當中，開始接受HAART治療後，第一次梅毒感染的平均年齡為31.8歲；開始接受HAART治療後，直到第一次梅毒感染的平均天數為385.5天 (圖四)；開始接受HAART治療後，90天內發生梅毒感染則有17位（8.6%）。

關於發生梅毒的相關行為因素目前仍持續使用個管資料分析中。

四、結論與建議

根據這個研究，我們發現在 2009 年到 2015 年間在台大醫院追蹤的愛滋病毒感染者梅毒發生率持續增加。雖然大多數人都有接受抗愛滋病毒治療愛滋病毒，愛滋病毒感染獲得適當控制，梅毒的風險依然沒有降低。我們的研究顯示至少 25%的感染者，尤其是

男同志，持續有高風險的性行為導致感染致梅毒。在感染梅毒時患者生殖器或者口腔肛門的潰瘍部位愛滋病毒量升高，容易造成傳染他們的性伴侶，同時他們也容易被傳染肝炎病毒或者抗藥性的愛滋病毒，造成慢性肝炎或者增加後續治療的困難度。因此在臨床照護上，除了持續維持服用愛滋病毒藥物遵囑性以外，持續提供資訊衛教維持安全性行為，也是長期愛滋病毒照護治療的重點。

對政策之具體建議:為降低愛滋病毒感染者的梅毒發生率,除了感染者服用愛滋病毒藥物外，應持續對感染者提供安全性行為的衛教資訊。

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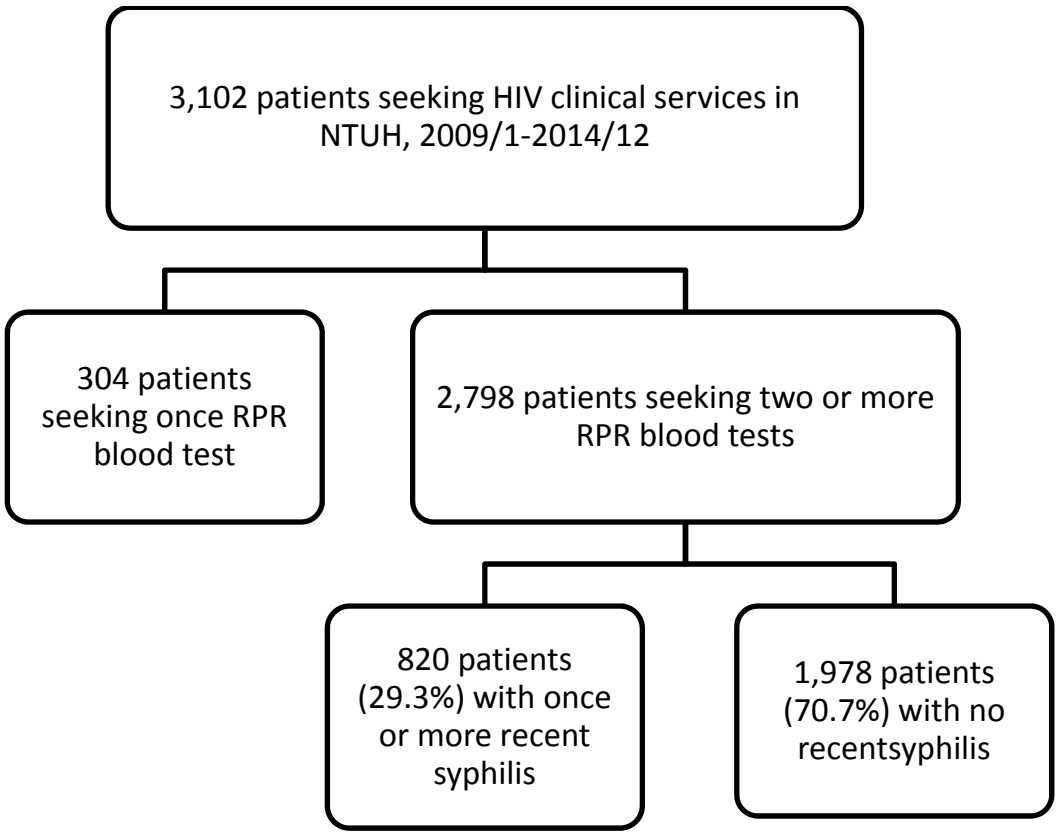
表一、Comparisons of characteristics between patients seeking HIV clinical services at NTUH with or without recent syphilis

Characteristics	recent syphilis	No recent syphilis	p Value
	N=820	N=1978	
Male, n (%)	**818 (99.8)**	**1847 (93.4)**	<.0001*
Age at the study started (mean ± SD), yr	**32.5 (±8.3)**	**36.7 (±11.0)**	<.0001*
Age at first syphilis in the study (mean ± SD), yr	**34.5 (±8.6)**	-	
Risk, n (%)			<.0001*
MSM	**716 (87.3)**	**1382 (69.9)**	
Heterosexual	**23 (2.8)**	**279 (14.1)**	
IDU	**8 (1.0)**	**69 (3.5)**	
Others	**73 (8.9)**	**248 (12.5)**	
Baseline lab results			
HBsAg positive, n (%)	**112 (13.7)**	**292 (14.8)**	0.4759
Anti-HBs positive, n (%)	**461 (56.2)**	**962 (48.6)**	0.0006*
Anti-HBc positive, n (%)	**234 (28.5)**	**535 (27.0)**	0.5583
Anti-HCV positive, n (%)	**60 (7.3)**	**183(9.3)**	0.1048
baseline RPR≧4, n (%)	**322 (39.3)**	**262 (13.3)**	<.0001*
baseline RPR≧32, n (%)	**146 (17.8)**	**132 (6.7)**	<.0001*
HCV infection after study started, n (%)	**86 (10.5)**	**40 (2.0)**	<.0001*
HCV infection after 1st syphilis occurred, n (%)	**68 (79.1)**	-	

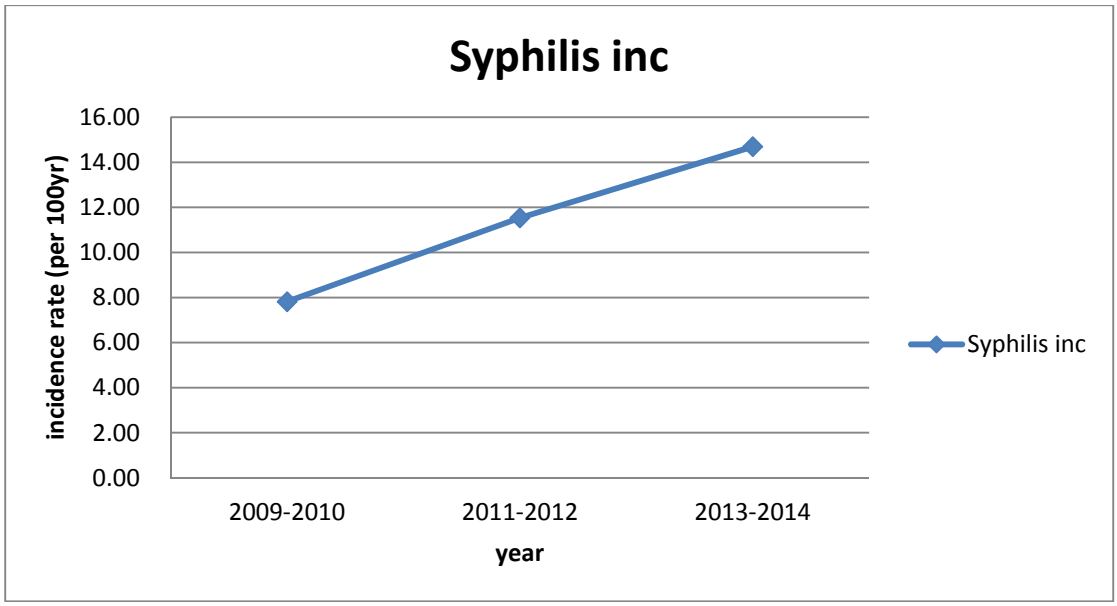
表二、Comparisons of characteristics between patients starting HAART at NTUH with or without recent syphilis

Characteristic	syphilis after HARRT	Without syphilis	P value
Case number at baseline, n=	**198**	**598**	
Age at HAART, mean (SD), yr	30.7 (6.8)	33.1 (9.1)	0.0008
< 20, n (%)	3 (1.5)	13 (2.2)	
20-29	96 (48.5)	245 (41.0)	
30-39	78 (39.4)	212 (35.5)	
40-49	21 (10.6)	103 (17.2)	
≧50	0 (0.0)	25 (4.2)	
Male, n (%)	198 (100.0)	577 (96.5)	0.0075
Risk factor, n (%)			0.0103
MSM	195 (98.5)	554 (92.6)	
Heterosexual	2 (1.0)	32 (5.4)	
IDU with sharing needles	1 (0.5)	12 (2.0)	
HBsAg-positive before HAART, n(%)	21/191 (11.0)	53/573 (9.3)	0.48
Anti-HCV-positive before HAART, n(%)	8/197 (4.1)	28/588 (4.8)	0.684
Baseline CD4 count on HAART, mean (SD), cells/μl	291 (159.0)	287 (191.1)	0.8189
Baseline CD4 count <350 cells/μl, n(%)	130 (65.7)	389 (65.1)	0.8766
Baseline PVL on HAART, mean (SD), log_{10} copies/ml	5.0 (0.7)	4.9 (0.7)	0.1458
Baseline PVL on HAART>100,000 copies/ml, n(%)	89 (45.0)	233 (39.0)	0.1369
Age at first syphilis, mean (SD), yr	31.8 (6.8)	-	-
Duration between HARRT starting and first syphilis, mean (SD), d	385.5 (249.9)	-	-
Duration < 90 days, n (%)	17 (8.6)		
PVL after on HAART 1 mo <50 copies/ml, n(%)	20/192 (10.4)	73/581 (12.7)	0.5223

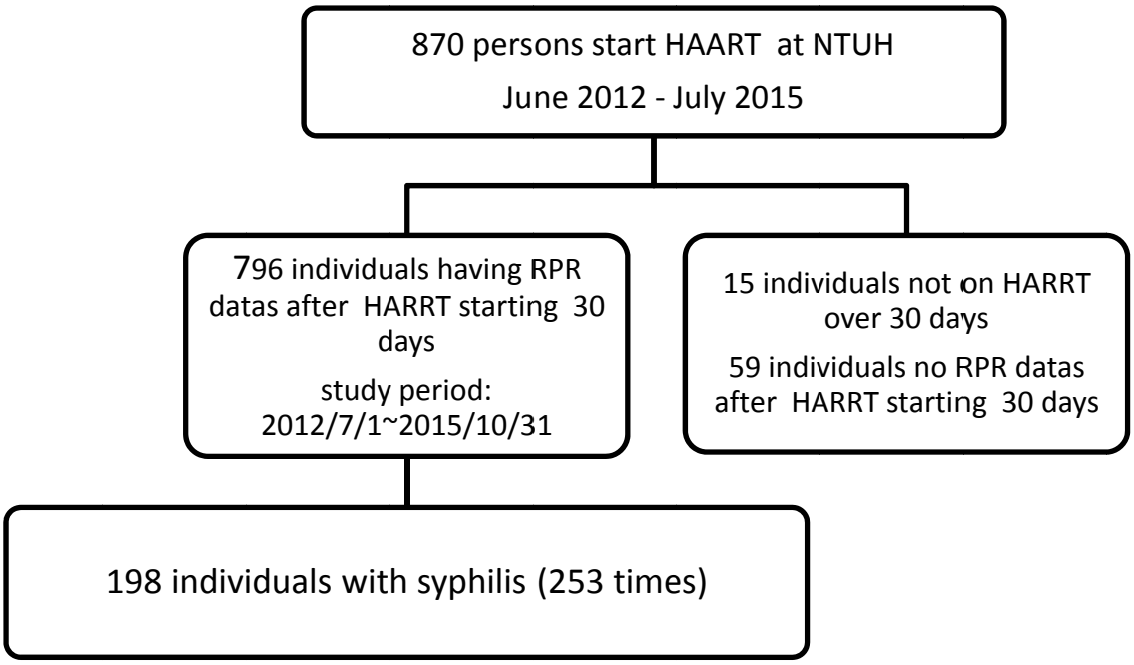
圖一、Surveillance study for syphilis in NTUH



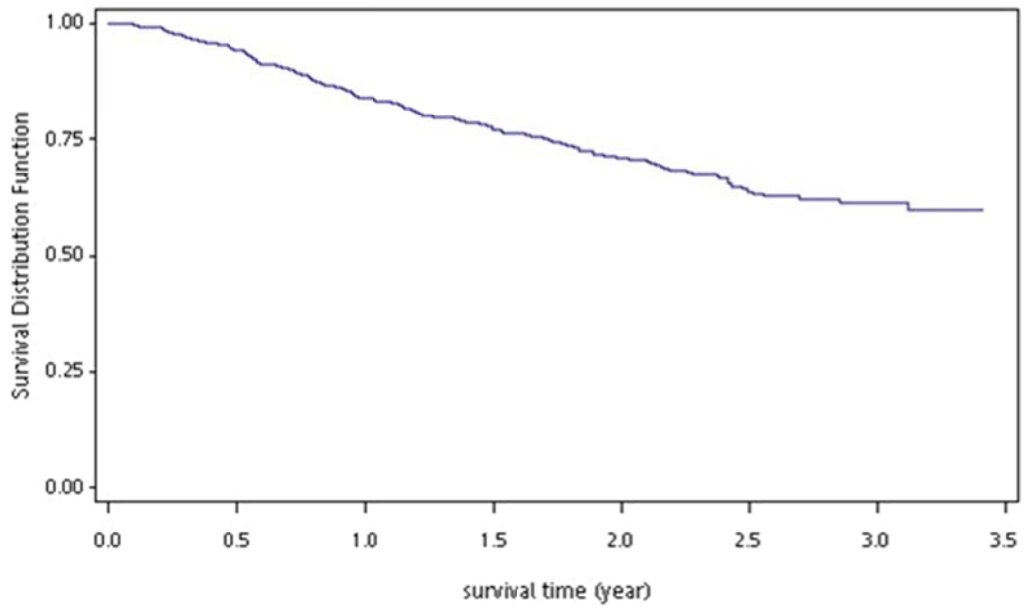
圖二、Syphilis incidence in NTUH during 2009-2014



圖三、愛滋病毒感染患者在開始接受抗病毒藥物後發生梅毒的研究流程圖



圖四、**Kaplan-Meir**圖顯示第一次梅毒的發生



附件十一

計畫編號：MOHW104-CDC-C-114-000301

衛生福利部疾病管制署 104 年委託科技研究計畫

計畫名稱：愛滋病毒感染者之糖尿病發生率及預後

年 度 研 究 報 告

執行機構：台大醫院 計畫

計畫主持人：蔡人植、巫沛瑩

研究人員：

執行期間： 104 年 1 月 1 日至 104 年 10 月 31 日

＊本研究報告僅供參考，不代表本署意見，如對媒體發布研究成果應事先徵求本署同意*

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摘要 (中文)

隨著愛滋病毒感染者使用抗愛滋病毒藥物延長生命後，愛滋病毒感染者因年齡的增加與老化,和長期使用抗愛滋病毒藥物,相繼發生的代謝相關併發症,包括葡萄糖耐受不良、糖尿病與脂肪代謝異常，這些往往都是心血管疾病的危險因子之一。糖尿病是長期的代謝疾病，與引起大小血管病變有相關，例如眼睛的視網膜病變、慢性腎臟病、心臟血管疾病、甚至是腦中風。因此,早期發現此疾病並控制此疾病，將會降低社會照護的成本。

在本研究中,我們採世代追蹤的研究方法，針對在 2004 年 1 月 1 日至 2013 年 12 月 31 日於本院新通報之個案且持續留在本院追蹤之個案。持續追蹤到 2015 年 10 月 31 日為止。我們估算糖尿病發生率的方面，並且嘗試找尋可能的相關因素。

在這研究期間,一共有 1,288 愛滋病毒感染病患在台大醫院就醫並且持續追蹤，總追蹤平均時間為 5.5 年，個案平均年齡為 32.9 歲，其中有 1,256 (97.5%)為男性，32 (2.5%)為女性。在總追蹤時間為 7,071 人年中，我們一共發現 24 位新發生糖尿病之個案，糖尿病的累計發生率為 3.39/1000 人年。我們將個案進入研究的時間分為三個時段，分別為 2004-2006 年(204 位)、2007-2009 年(337 位)、2010-2014 年(747 位)，所有時段都往後繼續觀察二年,在三個時段的糖尿病發生率分別為 1.4/1000 人年、2.6/1000 人年、1.2/1000 人年。

每一時段的觀察期,我們預計是五年。因此, 此研究將繼續三年,以利於找出相關因子。

關鍵字：愛滋病毒感染者、糖尿病發生率、抗愛滋病毒藥物

摘要 (英文)

With introduction of effective combination antiretroviral therapy, the survival of HIV-positive patients has significantly improved. Exposure to antiretroviral therapy, ageing and inflammation related to HIV replication may increase the risk of metabolic complications, including diabetes mellitus, hyperlipidemia, and lipodystrophy that leads to increased risk for cardiovascular diseases.

In this study, we included 1288 HIV-positive patient who sought HIV care and combination antiretroviral therapy at the National Taiwan University Hospital between 2004 and 2013. During the total observation duration of 7071 person-years, 24 cases of diabetes mellitus were detected, which accounted for an overall incidence rate of 3.39 per 1000 person-years. Three study periods were defined: 2004-2006 (n=204), 2007-2009 (n=337), and 2010-2014 (n=747), for which the observation duration for the patients in each study period was extended for 2 years. The incidence rate for the study year 2004-2006, 2007-2009, and 2010-2013 was 1.4 per 1000 person-years, 2.6 per 1000 persons-years and 1.2/1000 person-years, respectively. The observation will be extended, with the last included patient to complete at least 5 years of follow-up. The study will be continued to identify the associated factors with development of diabetes mellitus.

一、前言

自從雞尾酒療法於 1996 年問世後，大大改善了愛滋病毒感染者的存活率[1]。然而隨著愛滋病毒感染者使用抗愛滋病毒藥物延長生命後，研究人員開始觀察到愛滋病毒感染者因年齡的增加與老化，相繼發生的代謝相關併發症包括葡萄糖耐受不良、糖尿病與脂質代謝異常，這些往往都是心血管疾病的危險因子之一[2-5]。

葡萄糖耐受不良與糖尿病在有抗愛滋病毒藥物的時代已成為一個常見的問題，過去的研究當中發現使用蛋白酶抑制劑的病患，有 6-7%的糖尿病發生率[6]。糖尿病是長期的代謝疾病，與其他的大小血管有相關，例如眼睛的視網膜病變、慢性腎臟病、心臟血管疾病、甚至是腦中風，所以無法早期發現此疾病並控制此疾病，將會增加社會照護的成本。此外，過去的世代研究也發現使用抗愛滋病毒藥物的患者比起沒有使用藥物的病患或是未感染愛滋病毒的人，有較高的糖尿病發生率[7-9]。不過，在不同的世代研究當中，糖尿病發生率的變異差距甚大，從每 4.4 /1000 人年至每 47/1000 人年，這些差異也許來自於不同種族以及對於糖尿病診斷的定義有所不同。除了不同種類抗愛滋病毒藥物和糖尿病在愛滋病患的發生率有相關之外，在過去文獻當中，年紀、性別、肥胖、種族也跟新發生的糖尿病有相關[8-11]。這些都是在西方國家所發表的結果，疾病管制署羅一鈞醫師等已經發表的病例對照研究中，在 824 位愛滋病毒感染者當中，有 50 位新診斷糖尿病患者，發生率為 13.1 /1000 人年。與 100 位對照者相比，有糖尿病家族史、使用 zidovudine 與同時使用蛋白酶抑制劑的患者得到糖尿病的機會分別是 2.656 倍、3.168 倍與 2.528 倍[12]。再者，在台灣超過 40 歲的愛滋病毒感染族群中，我們也觀察到不同年齡層所發生的合併症，發現年齡大於 50 歲相比於年齡屆於 40-49 歲的愛滋病毒感染患者，有糖尿病的比率高出許多(18.4% vs 4.6%, P<0.0001)，此外，發生高血壓、高血脂與冠心病的比例都相對增高[13]。

台灣非愛滋病毒感染者得到糖尿病的發生率估計為 1.97/1000 人年[14]，而愛滋病毒感染者糖尿病的發生率高達一般族群的 6.6 倍，並且隨著年紀的上升，發生糖尿病的比例亦增[13]。這在存活率漸增的愛滋病毒感染者中是個重要的議題。在台灣過去的研究當中，雖知家族史、藥物是危險因子，仍然有些議題未被解決，且隨著新的抗愛滋病毒藥物的引進使用、愛滋病毒感染人數的增加，副作用較多的藥物已漸漸較少為醫師所使用，例如：stavudine、didanosine、indinavir 等，而新抗愛滋病毒藥物使用增加，例如 ataznavavir, raltegravir, maraviroc 等，這些因素在台灣過去的研究仍尚未被納入分析當中。

再者，發生糖尿病之愛滋病毒感染者的血糖控制、哪些因素會影響患者的血糖控制、發生糖尿病慢性併發症的機率等，這些都有待進行更深入的研究，來釐清與提供未來更進一步的資訊。以便臨床照護者在照顧感染者時有更多資訊可以參考，並提供更多本土的資料給健康政策制定的相關單位，以制定更符合台灣的相關健康政策。

二、材料與方法

我們採世代追蹤型研究方法，研究對象為 2003 年 1 月 1 日至 2013 年 12 月 31 日在本院新通報之個案且持續留在本院追蹤之個案。納入條件為大於十八歲以上於感染科門診追蹤之愛滋病毒感染患者，排除條件為在進入研究時已經診斷糖尿病之個案。

資料收集時間為 2003 年 1 月 1 日至 2013 年 12 月 31 日，之後再追蹤兩年，直到 2015 年 10 月 31 日為止。

基本資料收集(性別、年紀)、抗愛滋病毒藥物、過去共病史(包含梅毒、B 型肝炎、C 型肝炎、高血壓)、糖尿病相關慢性併發症。紀錄個案每年的之抽血數值變化包含飯前血糖(AC sugar)、糖化血色素(HbA1c)、血脂肪(TG、T-CHO、HDL、LDL) 、愛滋病毒量、CD4 淋巴球計數。

糖尿病的診斷定義為，飯前空腹血糖≧126 mg/dl 且糖化血色素＞6.5%。

計算發生率的方面，我們是以人年(person-year)來計算並呈現追蹤的資料，比較有無發生糖尿病與其他因子之間的關係，我們會使用存活率分析方法(Cox proportional hazards model)分析哪些因子與糖尿病發生率有相關。我們所有的統計都是使用 SAS (Version 9.3)。

三、結果

從2003年1月1日至2013年12月31日在本院新通報之個案為1,712人，排除失聯個案為406人，新通報個案同時有糖尿病時的個案為6人，最後符合收案之個案為1,288人，這1,288位個案在同時追蹤至2015年10月31日為止。收案流程圖(圖一)

在1,288位個案中，總追蹤時間為7,071人年，共發現24位新發生糖尿病之個案，糖尿病的發生率為3.39/1000人年，總追蹤平均時間為5.5年，個案平均年齡為32.9歲，其中有1,256 (97.5%)為男性，32 (2.5%)位為女性。242 (18.8%)位在新診斷時同時有伺機性感

染，161 (12.6%)位為B型肝炎表面抗原反應為陽性，81 (6.3%)位的C型肝炎抗體呈現陽性。在基礎點的CD4平均值為287 (cell/μL)，基礎點的病毒量平均值為4.88 log_{10}(copies/mL)，基礎點的飯前空腹血糖為88 mg/dl。(表一)

我們將個案進入研究的時間分為三個時段，分別為2004-2006年(204位)、2007-2009年(337位)、2010-2014年(747位)，三個時段的發生率分別為1.4/1000人年 (1/717)、2.6/1000人年 (3/1167)、1.2/1000人年 (3/2620) (圖二)。

三個時段的追蹤平均年為3.5年，我們發現在2010-2014年診斷的個案相較於2004-2006年診斷的個案平均年齡較為年輕 31 vs. 36歲；基礎點的CD4，2010-2014年診斷的個案相較於2004-2006年診斷的個案平均數值較高 311 vs.196 cells/μL，基礎點的病毒量，2010-2014年診斷的個案相較於2004-2006年診斷的個案平均數值較低 5.1 vs.4.8 log_{10} copies/mL。(表二)

四、討論

在我們的研究裡發現愛滋病毒感染者的糖尿病累計發生率為3.39/1000人年，此發生率與一個研究結果相似為瑞士(4.4/1000人年) [9]，相較於其他研究較低分別為泰國的研究(5.0/1000人年) [15]、D:A:D 研究團隊(5.7/1000人年) [11]、美國的研究(47/1000人年) [8]、美國針對女性的大型研究(25/1000人年) [10]以及台灣羅一鈞的案例對照研究(13.1/1000人年) [12]。

本研究與其他研究結果的差異可能是我們的研究族群平均年齡較輕(32.9 vs 39-51歲) [11,12,15-17]、男性佔多數(97.5% vs 56-73%)[11,15-17]。本研究與兩個研究結果差異不大的可能原因是年齡較相近 [9,15]。

本研究尚未分析個案服用抗病毒藥物的種類、時間，所以我們暫時無法知道藥物對於糖尿病可能的影響，在未來我們會再加入抗病毒藥物的種類及時間以增加資料的完整性。在國外的研究裡有發現長期使用抗病毒藥物也會增加代謝疾病的風險,因此我們將繼續觀察三年,以期能較精確推估發生率和發現其他相關因子。

五、結論與建議。

本研究結果共追蹤了7,071人年，共發現24位新發生糖尿病之個案，糖尿病的發生率為3.39/1000人年，研究期間分為三個時段，分別為2004-2006年、2007-2009年、2010-2014

年，三個時段的發生率分別為1.4/1000人年、2.6/1000人年、1.2/1000人年。

六、重要研究成果及具體建議

愛滋病毒感染者在隨著抗病毒藥物的使用之後，感染者存活率提高且生命期也延長，感染者年齡也越來越長，所以慢性病(糖尿病、高血壓、腎臟疾病、骨質疏鬆、代謝症候群)發生的比例也越來越高，長期追蹤型的研究是未來的必然趨勢。

本研究為一個世代追蹤型研究，總共追蹤12年，其研究的結果可以提供愛滋病照護在照護慢性病時的重要參考指標，提醒臨床照護者，糖尿病在感染者也是需要被關注的議題。

對政策的具體建議:愛滋病毒感染者慢性病(糖尿病、高血壓、腎臟疾病、骨質疏鬆、代謝症候群)發生的比例越來越高，需要定期且長期追蹤。

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圖一 收案流程圖

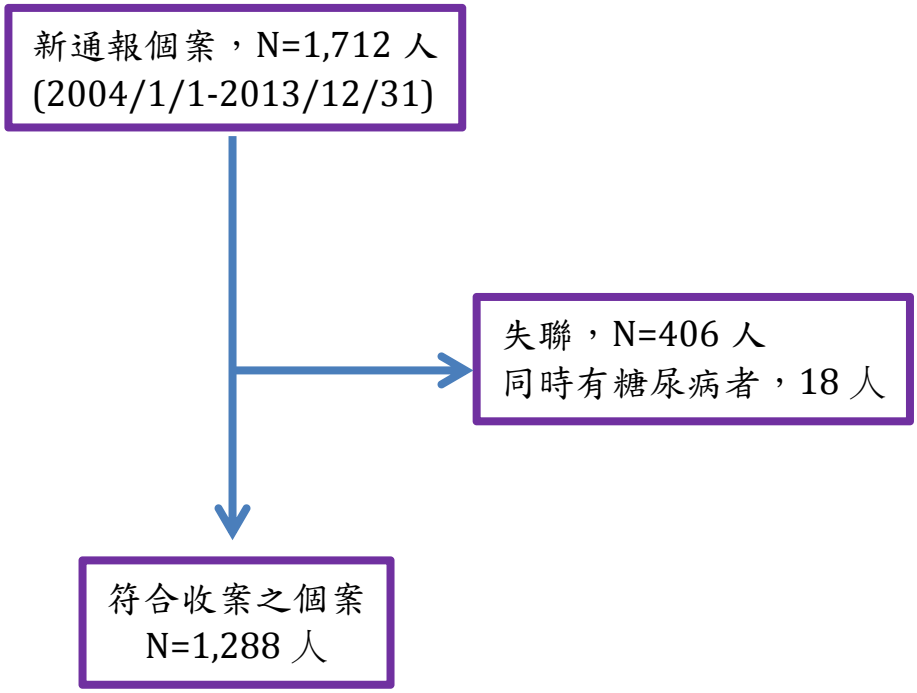


Figure 2. Calendar incidence of new-onset diabetes

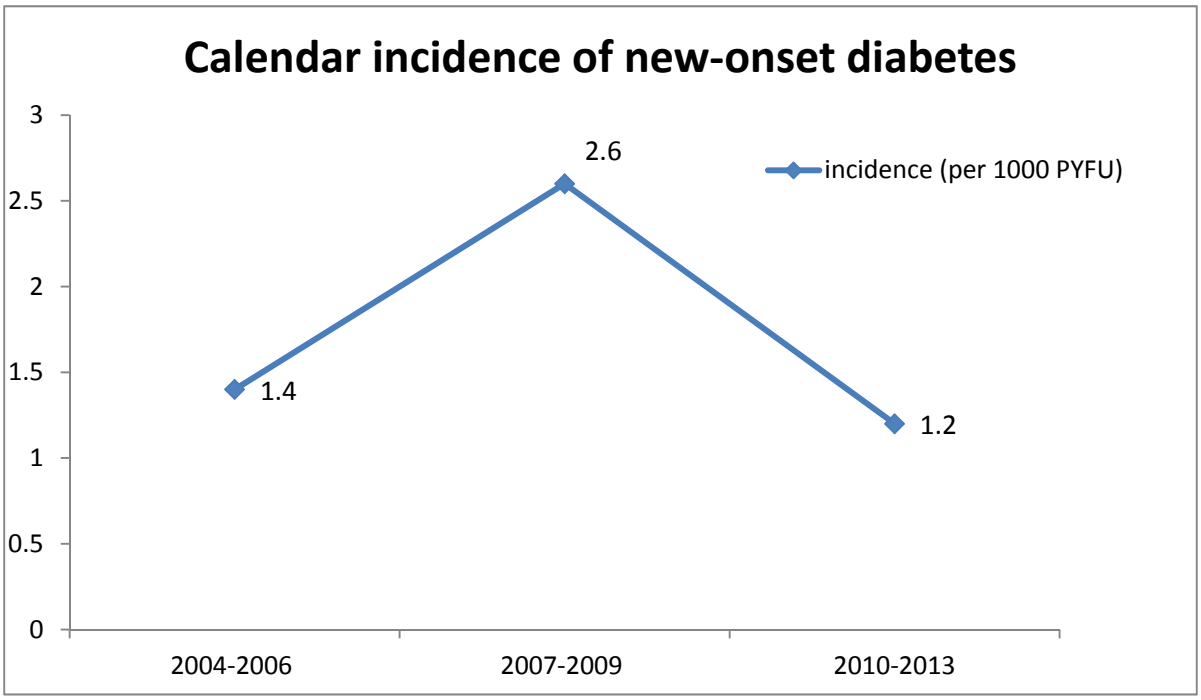


Table 1. Characteristics of HIV-infected cohort by incident diabetes mellitus

Variable	No diabetes N=1264 (98.1)	With diabetes N=24 (1.9)	All subjects N=1288	HR (Hazard ratio) (95% CI)	P-value
Gender Female Male	 31 (2.45) 1233 (97.55)	 1 (4.17) 23 (95.83)	 32 (2.48) 1256 (97.52)	0.50 (0.04, 5.83)	0.58
Age in year, mean (SD)	32.673 (9.20)	42.26 (10.73)	32.85 (9.32)	1.05 (0.99, 1.11)	0.13
Duration of follow-up, mean-year (SD)	5.47 (2.79)	6.47 (2.936)	5.49 (2.79)		
duration of diabetes ,month, mean (SD)	-	155.8 (71.41)	-	-	-
First enrolment year 2004-2006 2007-2009 2010-2013	 188 (14.60) 332 (26.27) 744 (58.86)	 16 (66.67) 5 (20.83) 3 (12.50)	 204 (15.84) 337 (26.16) 747 (58.00)	-	 <.0001 0.5486 <.0001
OI	235 (18.59)	7 (29.17)	242 (18.79)	0.76 (0.17, 3.37)	0.72
Cancer	18 (1.42)	2 (8.33)	20 (1.55)	0.03 (0.001, 1.06)	0.03
HBsAg-positive, n (%)	155 (12.30)	6 (26.09)	161 (12.55)	3.20 (0.67, 15.25)	0.14
Positive anti-HCV antibody, n (%)	80 (6.35)	1 (4.35)	81 (6.32)	0.81 (0.08, 7.91)	0.85
Hypertension	45 (3.56)	9 (37.50)	54 (4.19)	5.95 (1.25, 28.29)	0.025
CAD	10 (0.79)	2 (8.70)	12 (0.93)	6.17 (0.89, 42.79)	0.065
Baseline CD4 count (cell/μL)	289.9 (247.0)	179.2 (219.5)	287 (246)	0.99 (0.99, 1.004)	0.601
Baseline HIV-RNA (copies/mL)	4.88 (0.90)	5.212 (0.65)	4.88 (0.90)	0.71 (0.26, 1.91)	0.49
Baseline fast glucose, mean (SD), (mg/dl)	88.54 (9.82)	104.4 (27.91)	88.8 (10.5)	1.08 (1.04, 1.13)	0.0002

Table 2 Characteristics of HIV-infected cohort by enrolment year

Variable	First enrolment year 2004-2006, N=204	First enrolment year 2007-2009, N=337	First enrolment year 2010-2014, N=747	All subjects N=1288
New onset diabetes, n/1000 PYFU	1.4/1000 PYFU (1/717=1.4)	2.6/1000 PYFU (3/1167=2.6)	1.2/1000 PYFU (3/2620=1.2)	3.39/1000 PYFU (24/7071=3.39)
Female Male	12 (5.9) 192 (94.1)	9 (2.7) 328 (97.3)	11 (1.5) 736 (98.5)	32 (2.48) 1256 (97.52)
Age in year, mean (SD)	36 (10)	33 (9.2)	31 (8.9)	32.85 (9.32)
Duration of follow-up, mean-year (SD)	3.5 (0.8)	3.5 (0.8)	3.5 (1.28)	5.49 (2.79)
OI	58 (28.4)	73 (21.7)	111 (14.9)	242 (18.79)
Cancer	3 (1.4)	7 (2.1)	10 (1.3)	20 (1.55)
HBsAg-positive, n (%)	30 (14.7)	52 (15.4)	79 (10.5)	161 (12.55)
Positive anti-HCV antibody, n (%)	20 (9.8)	23 (6.8)	38 (5.1)	81 (6.32)
Hypertension	25 (12.3)	20 (5.9)	9 (1.2)	54 (4.19)
CAD	7 (3.4)	4 (1.2)	1 (0.13)	12 (0.93)
Baseline CD4 count (cell/μL)	196 (220)	290 (290)	311 (226)	179.2 (219.5)
Baseline HIV-RNA (copies/mL)	5.1 (0.7)	4.9 (0.9)	4.8 (0.9)	5.212 (0.65)
Baseline fast glucose, mean (SD),(mg/dl),N=105,232,557	88 (16)	90 (11.2)	88 (8.7)	104.4 (27.91)
Baseline HbA1C mean (SD), (%),N=0,5,40		5.9 (0.7)	5.4 (0.3)	5.4 (0.4)

附件十二

計畫編號：MOHW104-CDC-C-114-000301

衛生福利部疾病管制署 104 年委託科技研究計畫

愛滋感染者帶狀皰疹發病率和水痘—帶狀皰疹病毒血清陽性率

年 度 研 究 報 告

執行機構：台大醫院雲林分院

計畫主持人：林綺英

研究人員：

執行期間：104 年 1 月 1 日至 104 年 12 月 31 日

＊本研究報告僅供參考，不代表本署意見，如對媒體發布研究成果應事先徵求本署同意*

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中文摘要

帶狀疱疹(俗稱皮蛇, HZ)是 VZV(水痘-帶狀疱疹病毒)再度活化的表現，這種病毒在初次感染時會引發水痘。初次感染之後，此病毒會潛伏在背根神經節或腦感覺神經節中，直到再度活化引發帶狀疱疹，可以是一個使人衰弱的疾病。 在有高效能抗病毒藥物(HAART)之前，相較於非愛滋病毒感染者，愛滋病毒感染病患帶狀疱疹的整體年齡調整後的發病率約有 12-17 倍之高。 HAART 減少了最常見的伺機性感染的發生率; 然而，一些研究發現，它不能完全消除發生帶狀疱疹的風險，原因是由於 HIV 感染改變了細胞免疫。最近來自美國和德國的流行病學調查估計，接受抗病毒藥物的愛滋病毒感染者感染帶狀疱疹的風險仍然比一般人群高出 4-11 倍。台灣最近沒有這樣的流行病學調查。目前現有的流行病學調查是過去 1994-2003 年時所做的，那時是早期 HAART 時代，愛滋病毒感染者在台灣地區帶狀疱疹的發病率為 5.05 每 100 人年。更新愛滋病毒感染者之帶狀疱疹發生率和血清陽性率的研究是必要的，以確定長期使用 HAART 是否可進一步降低愛滋病毒感染者的帶狀疱疹發病率，並且評估是否可發展出針對愛滋病毒感染者帶狀疱疹的預防策略，這是值得研究的。

此外，以往的研究顯示，和免疫功能正常的宿主相較，愛滋病毒感染病患帶狀疱疹表現往往較俱獨特性且病情嚴重，通常較難以處理。愛滋病毒感染病患往往有反覆帶狀皰疹發作，多個皮節被影響，及同時有神經系統的併發症，如帶狀皰疹後神經痛和帶狀皰疹眼症。有鑒於愛滋病毒感染病患罹患帶狀疱疹較容易有併發症，且易發生於較年輕的族群，目前還不知道是否有更多積極的具體策略適用於現今逐漸老化的愛滋病毒感染病患，雖然他們有較高的 CD4 淋巴細胞計數。

而對一般大眾而言，帶狀疱疹主要預防的策略是通過疫苗接種，以提高患者的免疫力，所以檢測愛滋病毒感染病患是否已俱有 VZV-IgG 抗體，是找出有罹患帶狀疱疹風險的愛滋病毒感染病患必要步驟。 Zostavax（減毒活病毒疫苗，比水痘疫苗 Varivax 多 14 倍的 Oka 株病毒）可以安全而有效地使用在一般民眾身上，而在免疫缺乏患者身上是否能安全使用，則需要後續更多研究之證實，或期待後續改良疫苗之成效呈現。

關鍵詞：帶狀疱疹(HZ) ， 人類免疫不全病毒(HIV) ，水痘-帶狀疱疹病毒(VZV)

英文摘要

Herpes zoster (HZ) is the clinical manifestation resulting from the reactivation of the varicella-zoster virus (VZV) and can be a debilitating illness. The overall age-adjusted incidence rates of HZ is estimated to be 12-17 fold higher in HIV-infected compared to HIV-uninfected persons before highly active antiretroviral therapy (HAART).[1] HAART has reduced the incidence of the most common opportunistic infections; however, some studies indicated that it cannot completely eliminate the increased risk of HZ due to altered cell-mediated immunity.[2] Recent epidemiological studies from the United States of America and Germany estimates that HIV-infected patients on modern ART still have a 4-11 fold increase in incidence rate of HZ compared to the general population.[3,4] There is no such recent data for HIV-infected patients on cART in Taiwan. The last epidemiological survey among HIV-infected patients in Taiwan was conducted between 1994-2003, in which the incidence of HZ in the early post-HAART era was 5.05 per 100 person-years.[5] An updated incidence and seroprevalence study is necessary to document whether long-term HAART has further reduced the incidence of HZ in this population, and thus whether, HIV patient-specific HZ preventative strategies are worthwhile and feasible.

In addition, previous studies have suggested that HIV patients manifest uniquely dramatic and protracted HZ infections compared with the immunocompetent host. HIV patients tend to have recurrent zoster episodes, multi-dermatomal involvement, and neurological complications such as post-herpetic neuralgia and zoster ophthalmicus.[1] It is not known whether more aggressive HIV patient-specific strategies, which recognize that complications occur at a much higher rate and among a much younger HIV population, remains relevant in a contemporary but aging HIV-infected population with higher baseline and aggregate CD4 lymphocyte counts.

As the major preventative strategy of HZ in the general population is to boost patients' natural immunity by vaccination, evidence of VZV-IgG antibody is necessary to define the HIV-infected population at risk of reactivation of VZV. Zostavax (a live-attenuated viral vaccine, with 14-fold more plaque forming units of the OKA strain virus than the chickenpox vaccine, Varivax) is safely and efficaciously used in general population. The use of Zostavax in immunocompromised patients needs further evidence to support the safety concern. The modified vaccine is possible to use in these patients in the furture.

keywords：herpes zoster, HIV infection, AIDS

一、前言

台灣 1994-2003 年間愛滋病毒感染者的帶狀疱疹的發生率為 5.05 每 100 人年，而根據根據全民健保資料，在 2000 年一般民眾帶狀疱疹發生率為是 4.45 每 1000 人年。[5-7] 台灣試驗性的小兒水痘免疫接種一開始僅在有限的地區推行，台灣自 2004 年開始推展全國性綜合疫苗注射。因此 2000～2003 年是預防接種前期，2004～2005 年是早期的水痘疫苗接種時期，而在 2006～2009 年是全疫苗接種期間，當時水痘疫苗平均接種率高達約 90％ 。在台灣所有孩童都需要在 12 個月前完成免費定期注射疫苗。

HAART 不能完全消除愛滋病毒感染者罹患帶狀疱疹和其併發症的風險。最近來自美國和德國的流行病學調查估計，愛滋病毒感染者感染帶狀疱疹的風險仍比一般民眾高出 4-11 倍。目前台灣沒有這樣的流行病學調查。沒有愛滋病毒感染者在使用雞尾酒療法下，帶狀疱疹的發生率。此外，沒有愛滋病毒感染者水痘血清陽性率的相關研究。

本研究是為找出即使在使用 HAART 的情況下，仍因 HIV 感染導至細胞免疫受損的愛滋病毒感染者，發生帶狀疱疹的發生率，從而瞭解接種帶狀皰疹疫苗是否可能對這些人有益。

二、材料與方法

在 2015 年一月至二月間，設計標準的病例報告表。

收案定義為：從未接受過雞尾酒療法之愛滋病毒感染個案在追蹤時間內開始服用抗病毒藥物，並後續有在本院門診定期規則回診者。

研究分析期間為：自 2012 年 06 月 01 日至 2014 年 11 月 30 日為止

針對這些個案進行病歷回顧以及使用 LINE 詢問病患狀況，採用制式 EXCEL，記錄病患發生 Herpes Zoster(HZ)之時間，病況及相關治療，定期 CD4 數目以及血液愛滋病毒量數目，雞尾酒療法之使用時間，病患是否合併 B 型或 C 型肝炎感染， 並分析資料與計算發生率。

三、結果

自 2012 年 06 月 01 日至 2014 年 11 月 30 日為止，共 573 位從未接受過雞尾酒療法之愛滋病毒感染個案在此追蹤時間內開始服用抗病毒藥物，並在研究期間有定期追蹤回診，個案回報或經醫師診療是否有發生 Herpes Zoster，將其納入本研究發生率之計算與分析，資料之最後追蹤時間為 2015 年 01 月 31 日。

在這 573 位愛滋病毒感染患者中，主要為男性病患(560 位)，愛滋感染之主要風險為同性間性行為(535 位,93.4%) (附件 1-1)，其中有 33 位感染者在研究期間內發生 Herpes Zoster，發生率為每 100 人年 3.86 (總觀察人年為 854.19)。發生 Herpes Zoster 者主要為男性病患且為同性戀者(32 位)，平均年齡為 34 歲。

把有發生 Herpes zoster 之 33 位感染者與沒有發生 Herpes zoster 之 540 位感染者分為兩組作比較，發現在研究期間內有發生 Herpes zoster 之感染者在開始服用抗病毒藥物前有較低之 CD4 數目(CD4 : 157 VS 277 cells/ul，$p<0.17$)，較高之血液愛滋病毒量(PVL: 5.17 VS 4.86 log10 copies/ul, $p<0.002$)，以及在服用抗愛滋病毒藥物後有較高幅度之 CD4 數目之增加(CD4 增加: 114 VS 90.29 cells/ul, $p<0.027$)。(附件 1-2)

四、結論與建議

本計畫之結論與建議如下：

(1) 在有雞尾酒療法之時代，愛滋感染者發生帶狀疱疹的發生率比起過往已經有降低之情形，但仍比一般非感染者之發生率為高。

(2) 在開始服用雞尾酒療法之愛滋感染者中，若感染者在開始服用抗病毒藥物前有較低之 CD4 數目，較高之血液愛滋病毒量，以及在服用抗愛滋病毒藥物後有較高幅度之 CD4 數目之增加者，都有較高之機會發生帶狀疱疹。可考慮針對有以上特性之感染者，加強教育以及提醒，讓感染者更瞭解要甚麼情形要及早注意回診，接受適當之治療，避免發生併發症。

對政策之具體建議:服用抗病毒藥物前有較低之 CD4 數目者、較高之血液愛滋病毒量者、以及在服用抗愛滋病毒藥物後有較高幅度之 CD4 數目之增加者，應給予帶狀疱疹發生率較高的提醒。

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附件一~1

愛滋感者之臨床資料: 比較發生帶狀疱疹以及沒有發生帶狀疱疹之特性

Variable	All patients	Patients with zoster	Patients without zoster	P value
Patient No.	573	33	540	
Age, median (range), years	31 (17-80)	34 (22-61)	31 (17-80)	0.017
Male, n (%)	560 (97.7)	32 (97.0)	528 (97.8)	0.541
Risk of HIV infection, n (%)				
Homosexuals	535 (93.4)	32 (97.0)	503 (93.1)	0.999
Heterosexuals	27 (4.7)	1 (3.0)	26 (4.8)	
IDU	10 (1.7)	0 (0)	10 (1.9)	
Others	1 (0.2)	0 (0)	1 (0.2)	
Positive HBsAg, n (%)	62 (11.1)	5 (15.6)	57 (10.8)	0.383
Positive anti-HCV, n (%)	24 (4.3)	0 (0)	24 (4.5)	0.435
Prior zoster, n (%)	42 (7.3)	6 (18.2)	36 (6.7)	0.034
Duration from ART initiation to the development of zoster, median (range), days	128 (3-605)	128 (3-605)	NA	NA
<1 months of ART use, n (%)	5 (15.2)	5 (15.2)	NA	NA
1~3 months of ART use, n (%)	9 (27.3)	9 (27.3)	NA	NA
3~6 months of ART use, n (%)	5 (15.2)	5 (15.2)	NA	NA
6~12 months of ART use, n (%)	9 (27.3)	9 (27.3)	NA	NA
>12 months of ART use, n (%)	5 (15.2)	5 (15.2)	NA	NA
Baseline CD4, median (range), cells/uL	272 (0-888)	157 (15-627)	277 (0-888)	0.017
<200 cells/uL, n (%)	188 (33.0)	18 (56.3)	170 (31.6)	0.004
Baseline PVL, median (range), log_{10} copies/uL	4.86 (2-7)	5.17 (4-7)	4.84 (2-7)	0.002
>5 log10 copies/uL	231 (40.5)	21 (63.6)	210 (39.0)	0.005
CD4 1-month post-ART, median (range), cells/uL	379 (6-1154)	313 (6-1002)	384 (8-1154)	0.132
<200 cells/uL, n (%)	99 (18.2)	9 (28.1)	90 (17.5)	0.132
CD4 increase 1-month post-ART, median (range), cells/uL	**92.95 (-545~681)**	**114 (-67~647)**	**90.29 (-545~681)**	**0.027**
PVL 1-month post-ART, log_{10} copies/uL	2.56 (1-6)	3 (1-4)	2.54 (1-6)	0.017
>5 log10 copies/uL	6 (1.1)	0 (0)	6 (1.2)	0.999
Death, n (%)	8 (1.4)	0 (0)	8 (1.5)	0.620

附件一~2

比較發生帶狀疱疹以及沒有發生帶狀疱疹之感染者之多變數分析:

Variable	Reference	OR	95% CI	P value
Age	Per 10-year increase	1.301	0.909 – 1.861	0.151
Prior zoster	No prior zoster	2.928	1.103 – 7.769	0.031
Baseline CD4 counts	Per 50 cells/uL increase	0.884	0.783 – 0.998	0.047

附件十三

計畫編號：MOHW104-CDC-C-114-000301

衛生福利部疾病管制署 104 年委託科技研究計畫

計畫名稱：愛滋病毒感染患者接種 A 型肝炎疫苗後長期血清抗體效價研究

年 度 研 究 報 告

執行機構：國立台灣大學醫學院附設醫院

計畫主持人：洪健清

研究人員：洪健清、劉玟君

執行期間： 104 年 1 月 1 日至 104 年 10 月 31 日

＊本研究報告僅供參考，不代表本署意見，如對媒體發布研究成果應事先徵求本署同意*

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中文摘要

愛滋病毒感染者是感染 A 型肝炎病毒的高風險群。愛滋病毒感染者如果感染急性 A 型肝炎病毒，病毒血症和糞便中排出病毒的時間都較一般非愛滋病毒感染者高，這對於周遭親密接觸的朋友或親人，都會造成感染的風險。如果愛滋病毒感染者同時合併慢性 B 肝或者 C 肝炎病毒感染，再感染 A 型肝炎病毒後，發生急性肝臟功能代償失調的風險大大地增加。因此，愛滋病毒感染者是被建議接種 A 型肝炎疫苗的對象之一。在西元 2009-2010 年期間，我們在台大醫院針對愛滋病毒感染(N=365)和未感染愛滋病毒(N=217)的男同性戀提供 A 肝疫苗接種。接種後追蹤到 72 周的結果已經發表(Tseng YT, et al. Hepatology 2013)。追蹤到 48 周，我們發現愛滋病毒感染者儘管接種三劑的 A 肝疫苗，其血清抗體陽轉率依然低於非愛滋病毒感染者(75.7-77.8% vs. 88.5%)。再者，接種兩劑和三劑疫苗，對於愛滋病毒感染者的抗體陽轉率並沒有達到統計學上有意義的差異(75.7 vs. 77.8%)。接種疫苗時，有比較高 CD4 和血漿中檢測不到愛滋病毒的受試者會有較高的抗體陽轉率。這些愛滋病毒感染者，在完成接種試驗後，大多持續在本院的門診追蹤。本研究是針對已經完成接種後追蹤五年的受試者，再次檢測每一受試者在每一年抗體持續維持陽性反應的比例。我們計劃評估接種兩劑和三劑疫苗的愛滋病毒感染者，對於維持抗體陽性率，在追蹤五年後是否有差異。

關鍵字： 抗愛滋病毒療法; 疫苗接種; A 肝炎疫苗; 免疫力; 抗體反應

摘要（英文）

Most of hepatitis A virus (HAV) infections are self-limited; however, much more severe symptoms and complications associated with acute hepatitis A increase with age. In addition, persons with chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection who develop super-infection with HAV are at increased risk for fulminant hepatitis and associated mortality. In HIV-infected patients who acquire HAV, the duration of HAV viremia would be longer compared to that of the healthy persons, which may increase the risk of transmission to others. Therefore, HAV vaccination is recommended for HIV-infected patients, injecting drug users, men who have sex with men (MSM), hemophiliacs, patients with chronic liver disease, such as chronic HBV or chronic HCV infection, and persons planning to travel to or reside in endemic areas for HAV transmission. The response rate of HAV vaccination is almost 100% among HIV-uninfected persons who receive 2 standard doses of HAV vaccine. We recently enrolled 582 HAV-seronegative MSM for HAV vaccination, 365 HIV-infected (140 receiving 2 standard doses of HAV vaccine [1440 IU] and 225 3 doses) and 217 HIV-uninfected (receiving 2 doses). At 48 weeks of follow-up, the seroconversion rate remains lower in HIV-infected groups than HIV-uninfected groups (75.7-77.8% vs 88.5%). We have found that the seroconversion rate was significantly higher in those HIV-infected subjects who had higher CD4 counts and suppressed HIV replication with combination antiretroviral therapy. In this study, we aimed to compare the long-term persistence of HAV seropositivity and antibody concentrations between the HIV-infected subjected receiving 2 doses versus 3 doses of HAV vaccine 5 years following HCV vaccination.

Keywords: antiretroviral therapy; immunization; hepatitis A vaccine; immunogenicity; antibody response

一、前言

A 型肝炎病毒感染係經由糞口傳播，在衛生環境差的地方，自小孩開始即有受感染的風險，因此感染盛行率很高。隨著衛生環境改善和 A 型肝炎疫苗的接種施打，在資源豐富的國家地區，A 型肝炎病毒感染的發生率已經大幅下降，例如，美國自從 1995 年開始 A 型肝炎疫苗上市後，A 型肝炎病毒感染的年發生率已經從 1995 年的每十萬人有 12 例，降到 2009 年的 每十萬人有 0.6 例 (1)。在芬蘭，A 型肝炎病毒感染的年發生率已經從 1990 年的每十萬人有 3.6 例，降到 2007 年的 每十萬人有 0.3 例，再者大多數的案例都有國外相關的旅遊史 (2) 。

大多數 A 型肝炎病毒感染後，並不會造成嚴重明顯症狀。但是，隨著年紀增長，急性 A 型肝炎病毒感染可能會很嚴重，並產生併發症(3, 4)。其次，併有慢性 B、C 肝炎病毒感染的人，再感染 A 型肝炎病毒，發生急性肝炎和肝功能代償失調而致死亡的機會，大幅增加(5, 6)。在愛滋病毒感染者，如果感染 A 型肝炎病毒，發生病毒血症和糞便中排出 A 型肝炎病毒的時間都會延長，因此，會增加周遭親人朋友感染的風險(7)。因此，愛滋病毒感染者、靜脈藥癮者、血友病患者、慢性肝炎患者、打算到衛生條較差的地區旅遊或居住的人，都是被建議應該施打 A 型肝炎疫苗的對象 (8)。

一般人接種標準二劑 A 型肝炎疫苗產生抗體的機會，幾乎是百分之百(9,10)。但是，愛滋病毒感染者接種二劑疫苗(720 或 1440 ELISA units)產生抗體的機會大約是 68-88.2% ;如果接種三劑(720 ELISA units)，有文獻報告，陽性率可能提高到 76-90% (9-14)。再者，A 型肝炎疫苗接種後所產生的產生抗體效價(100-636 mIU/mL)，在愛滋病毒感染者相較於一般非感染者都來得低(>1000 mIU/mL) (9, 10)。最近，一個國際的隨機分組的臨床試驗研究顯示，接種三劑 A 型肝炎疫苗(1440 ELISA units)後，愛滋病毒感染者在追蹤達產生抗體的機會和抗體效價，似乎較接種二劑的感染者來得高，但是因為個案數較少，並未達到統計學上有意義的差異 (78.3% vs. 61.2%, *P*=0.07) (15)。

在西元2009-2010年期間，我們在台大醫院針對愛滋病毒感染(N=365)和未感染愛滋病毒(N=217)的男同性戀提供A肝疫苗接種，其中225位愛滋病毒染的男同志接種三劑，140位愛滋病毒染的男同志接種二劑。所有受試者接種後追蹤到72周的結果已經發表(16)。我們的結果顯示，追蹤到48周，愛滋病毒感染的男同志儘管接種三劑的A肝疫苗，其血清抗體陽轉率依然低於非愛滋病毒感染的男同志(75.7-77.8% vs 88.5%)。再者，接種兩劑和三劑疫苗，對於愛滋病毒感染的男同志的抗體陽轉率並沒有達到統計學上有意義的

差異(75.7 vs. 77.8%)。儘管，接種三劑的愛滋病毒感染的男同志的抗體效價，較接種二劑愛滋病毒感染的男同志來得高(2.29 ± 0.73 vs. 1.94 ± 0.66 $\log_{10}$ mIU/mL; $P < 0.01$)，但是依然比非愛滋病毒感染的男同志低(2.49 ± 0.42 $\log_{10}$ mIU/mL; $P < 0.01$)。我們同時發現，接種疫苗時，有比較高CD4 (adjusted odds ratio [AOR] for per 50 cells/lL increase, 1.13; 95% confidence interval [CI], 1.05-1.21)和血漿中檢測不到愛滋病毒 (AOR, 1.90; 95% CI, 1.10-3.28)的受試者會有較高的抗體陽轉率。這些愛滋病毒感染的男同志，在完成接種試驗後，大多持續在本院的門診追蹤。

本研究是打算針對已經完成接種後追蹤五年的受試者，再次檢測每一受試者在每一年抗體持續維持陽性反應的比例和定量其抗體效價。我們計劃評估在五年前接種兩劑和三劑疫苗的愛滋病毒感染者，對於維持抗體陽性率，在追蹤五年後是否有差異。

二、材料與方法

1.受試者納入條件

所有於 2009-2010 年間參加在台大醫院的 A 肝疫苗接種計劃，並且持續在本院追蹤之愛滋病毒感染者，並須在疫苗接種後第一年追蹤時產生抗體。如果未能產生抗體，我們將他們排除。

2. A 型肝炎抗體定量將採用 enzyme-linked immunosorbent assay (ELISA) (ETI-AB-HAVK PLUS; DiaSorin, Saluggia, Italy). 陽性的定義為 anti-HAV antibody >20 mIU/mL。

3.資料之蒐集處理評估

研究主持人和協同主持人採用制式的Excel紀錄病人資料。這些資料包括疫苗接種時、接種後每一年收集的檢體時間三個月內的最高的病毒量和最低的 CD4數目、肝功能、抗病毒藥物的種類等。 同時我們採用 GEE 的統計方式嘗試找尋影響受試者在第五年仍然維持有保護性抗體效價的因子。

我們對於資料分析的族群分成 intention-to-treat 和 per-protocol 兩群。

三、結果

1. 收案的流程圖和最後追蹤五年後的整體案數如圖一所顯示。
2. 在基礎點時，共有140人接種二劑和225人接種三劑HAV疫苗。
3. 依照intention-to-treat和per-protocol收納分析的個案數如圖一所示。

4. 隨著時間HAV抗體效價逐年下降，兩群受試者個別geometric mean concentration在每一年的boxplot 如圖二所示。下降趨勢並未達到統計學上有意義的變化。
5. 我們發現，在接種疫苗的第一年有產生抗體高於20 mIU/ml的受試者，經過五年後，根據intention-to-treat的分析方式，二劑和三劑依然能保有抗體數值高於20 mIU/ml的比例分別是 76.4% (84/110) 和78.9% (146/185) 。
6. 多變項的分析結果如表二所顯示。我們發現接種三劑的受試者較接種兩劑的受試者有8.09倍(95% confidence interval, 2.414-27.123)的機會在第五年追蹤時依然能維持抗體效價高於20 mIU/ml。

四、結論與建議

針對接受抗愛滋病毒藥物治療而且在基礎點呈現A型肝炎抗體陰性的愛滋病毒感染者施打三劑的A型肝炎疫苗，會較給予施打二劑疫苗，在完成五年的追蹤時，有八倍以上的機會，依然維持有高於20 mIU/ml的保護感染HAV的抗體效價。

五、重要研究成果及具體建議

近期國內發生愛滋病毒感染男同志的HAV群突發，在此報告完成時，已經有23位愛滋病毒感染的男同志被診斷為急性HAV感染。根據我們在台大醫院三年來新進開始接受抗愛滋病毒藥物的852位男同志的病歷資料調查，我們發現623位有檢驗HAV抗體，其中抗體呈陽性的比例僅有15.4%（n=96）。而沒有抗體的易感族群中，僅有13.1%接種了HAV疫苗。這個調查顯示，臨床醫師並沒有依照ACIP建議，積極檢測HAV抗體，對於抗體陰性的易感族群，HAV疫苗的接種率很令人擔心的低。接種率過低，可能源於醫師或病患對於急性HAV感染的病症大多不嚴重而且也不會有變成慢性的疑慮。其次，HAV疫苗價格高也是推廣易感受族群全面接種的一大障礙。再此，國際間對於HAV疫苗接種的長期追蹤資料也非常缺乏，現有資料無法提供臨床醫療人員或政策制定的公衛專家對於HAV疫苗接種建議的參考。

對政策之具體建議:在持續接受抗愛滋病毒藥物治療情況下，接種三劑的HAV疫苗較二劑能維持較持續的保護效果。

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Table 1. Baseline characteristics of HIV-positive patients receiving 2 or 3 doses of HAV vaccine

Characteristic	2-dose group	3-dose group	P value
Case number at baseline, n=	**140**	**225**	
Age, yr, mean (SD)	31.0 (5.3)	29.8 (5.0)	0.03
≦20, n (%)	2 (1.4)	4 (1.8)	
21-30	64 (45.7)	121 (53.8)	
31-40	74 (52.9)	100 (44.4)	
HBsAg-positive, n(%)	19/139 (13.7)	31/220 (14.1)	>0.99
Anti-HCV-positive, n(%)	8/140 (5.7)	12/223 (5.4)	>0.99
Baseline CD4 count, mean (SD), cells/μl	538 (227.9)	452 (199.5)	<0.01
Baseline PVL, mean (SD), log_{10} copies/ml	2.5 (1.2)	3.0 (1.4)	<0.01
HAART at vaccination, n (%)	94 (67.1)	131 (58.2)	0.12
CD4 count at 5 yr, mean (SD), cells/μl	655 (243.1)	603 (215.0)	0.07
PVL at 5 yr, mean (SD), log_{10} copies/ml	1.7 (1.0)	1.6 (0.9)	0.24

Table 2. Factors associated with persistent serological responses at 5 years after 2 or 3 doses of Hepatitis A vaccination

Variable	Reference	OR	95% CI	P - value
3 doses	2 doses	8.09	2.414-27.123	<0.001
Age at vaccination ≥ 30	Age <30	1.68	0.534-5.277	0.375
Treatment experienced	Not on cART	0.68	0.062-7.541	0.754
Baseline CD4 ≥350	Baseline CD4 <350	0.28	0.054-1.544	0.128
Baseline PVL ≥200	Baseline PVL <250	0.61	0.055-7.797	0.688
Chronic hepatitis B or C	No HBV/HCV	0.95	0.228-3.986	0.948
Smoker	Non-smoker	1.60	0.476-5.372	0.447
Time-updated infection with syphilis	No syphilis at any time points	2.55	0.550-11.826	0.232

Figure 1. Study flow

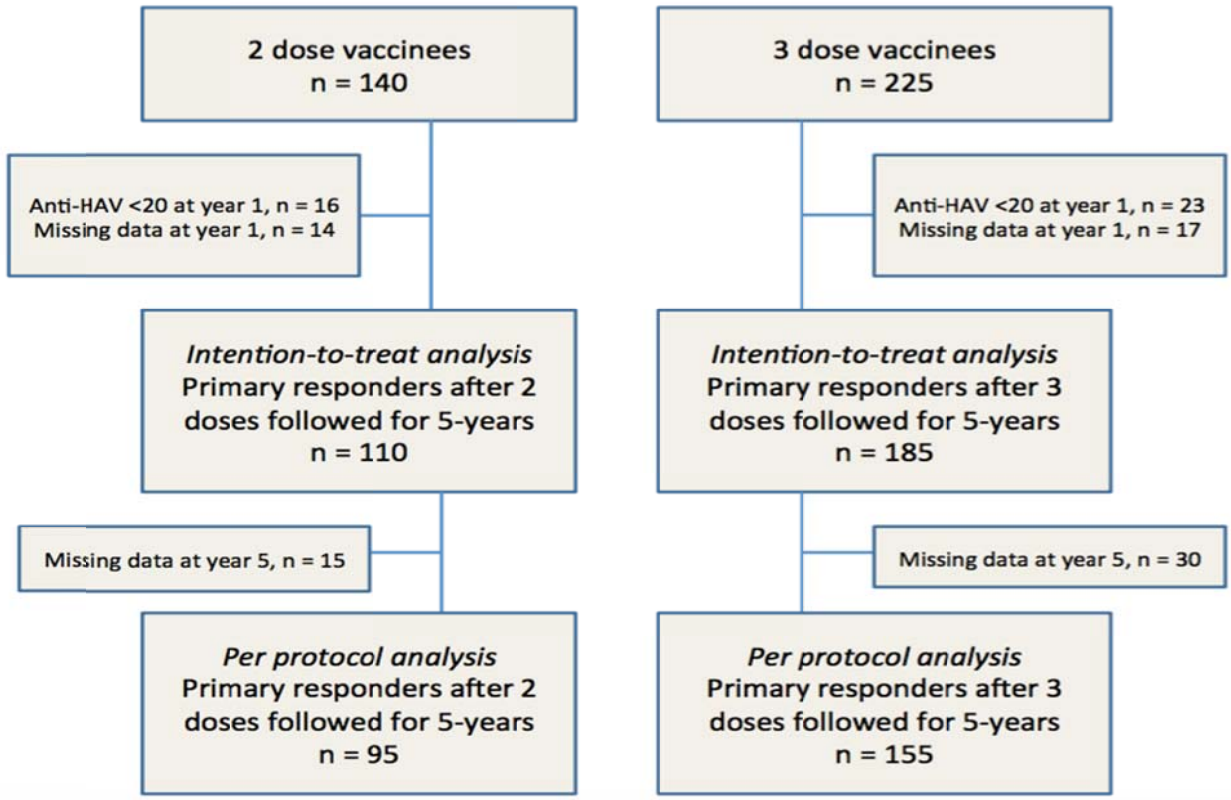
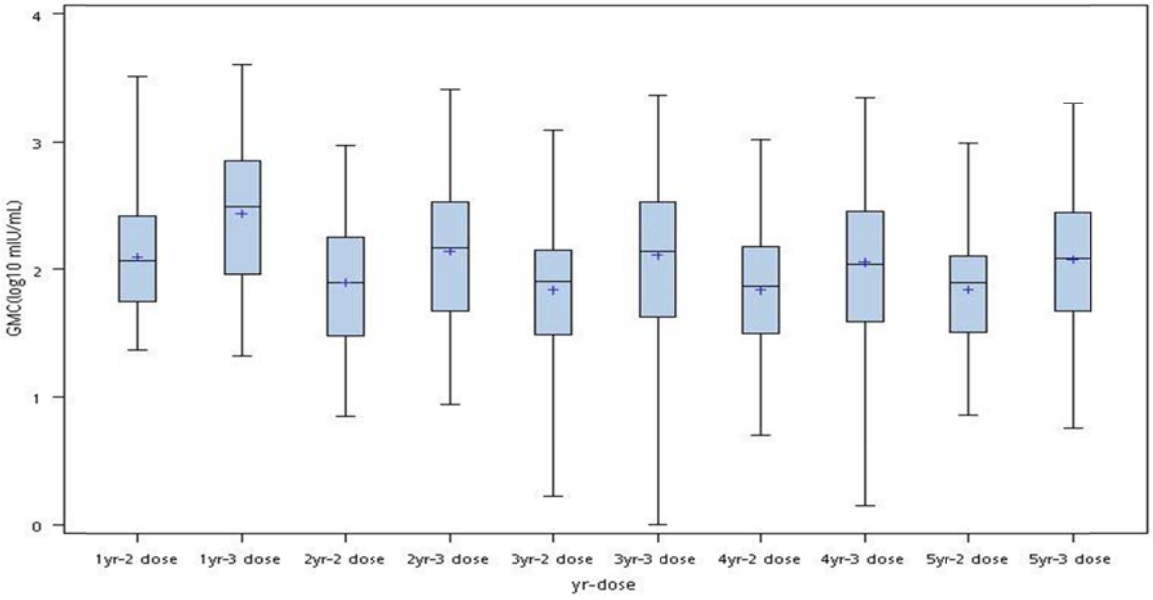


Figure 2. Serial GMCs of anti-HAV IgG in the 2- and 3- dose groups over 5 years



附件十四

計畫編號：MOHW104-CDC-C-114-000301

衛生福利部疾病管制署104年委託科技研究計畫

計畫名稱：接受全血丙型干擾素釋放分析檢測的 HIV 感染病患之結核病的發生率

年 度 研 究 報 告

執行機構：台大醫院 計畫主持人：洪健清

研究人員：孫幸筠

執行期間：104 年 1 月 1 日至 104 年 12 月 31 日

＊本研究報告僅供參考，不代表本署意見，如對媒體發布研究成果應事先徵求本署同意*

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中文摘要

617位曾檢測過丙型干擾素的HIV病毒感染病患中，排除九位在檢驗當下有活動性肺結核的病患(一位檢驗結果為陰性，八位檢驗結果為陽)，剩下608位檢測當下沒有活動性肺結核的病患中，有534位(87.8%)檢測結果為陰性，64位(10.5%)檢測結果為陽性，10位(1.6%)檢測結果為無法判定。檢測當下，534位檢測結果為陰性的平均CD4值為441.6 cells/μl，平均病毒量為2.89 $\log_{10}$ copies/mL; 64位檢測結果為陽性病患的平均CD4值為578.4 cells/μl，平均病毒量為2.35 $\log_{10}$ copies/mL，追蹤至2015/10/30止，檢測結果為陽性的病患中，僅一位未接受高效能抗病毒藥物治療。10位檢測結果為無法判定的病患，其平均CD4值為473.5 cells/μl，平均病毒量為2.79 $\log_{10}$ copies/mL。分析檢測結果陽性的相關因子為年紀較大者(調整後每增加十歲的勝算比為1.172, 95%的信賴區間為1.022-1.344, P=0.023)，過去有結核病病史者(調整後的勝算比為13.412, 95%的信賴區間為6.106-29.460, P<0.001)，及CD4值較高者(調整後每增加50CD4值的勝算比為1.062, 95%的信賴區間為1.017-1.109, P=0.007)。追蹤至2015/10/30止，僅一位檢測結果陽性患者沒有接受雞尾酒治療。另一位檢測結果陽性患者在追蹤337天後發生活動性結核病，在結核病診斷當下，病患已接受雞尾酒治療，當時的CD4為78 cells/uL，HIV病毒量為98 copies/mL。故64位檢測結果為陽性患者的結核病發生率為每100人年0.422件。 534位檢測結果為陰性，在追蹤1990人年後，沒有人發生結核病。24位檢測結果陽性且曾罹患過結核病的患者，在追蹤88人年後，沒有人發生結核病。在整體結核病發生率下降的台灣社會中，接受高效能抗病毒藥物治療的HIV病毒感染病患發生結核病的風險低。

英文摘要

Background: Interferon-gamma release assays (IGRAs) have been used to identify individuals at risk for developing active tuberculosis (TB). However, data regarding the risk of TB development in HIV-infected patients testing positive for IGRAs remain sparse in the era of combination antiretroviral therapy.

Methods: Between 2011 and 2015, 608 HIV-infected patients without active TB undergoing T-Spot.TB testing were enrolled in this prospective observational study at a university hospital designated for HIV care in Taiwan with a declining TB incidence from 72 per 100,000 population in 2005 to 53 per 100,000 population in 2012. All of the subjects were followed until Oct 30, 2015. The national TB registry was accessed to identify any TB cases among those lost to follow-up.

Results: T-Spot.TB tested negative in 534 patients (87.8%), positive in 64 patients (10.5%), and indeterminate in 10 patients (1.6%). In multivariate analysis, positive T-Spot.TB was significantly associated with older age (adjusted odds ratio [AOR], 1.172 per 10-year increase; 95% confidence interval [CI], 1.022-1.344, P=0.023), past history of TB (AOR, 13.412; 95% CI, 6.106-29.460, P<0.001), and higher CD4 counts at enrollment (AOR, per 50-cell/μl increase, 1.062; 95% CI, 1.017-1.109, P=0.007). Of the 64 patients testing positive for T-Spot.TB, none received isoniazid preventive therapy and all but 1 received combination antiretroviral therapy at the end of follow-up with the latest CD4 count and plasma HIV RNA load being 592.8 cells/μL and 1.85 $\log_{10}$ copies/mL, respectively. One patient (1.6%) developed active TB after 167 person-years of follow-up (PYFU), resulting in an incidence rate of 0.422 per 100 PFYU. None of the 534 patients testing negative for T-Spot.TB developed TB after 1990 PYFU, nor did the 24 patients with old TB and positive T-Spot.TB tests develop TB after 88 PYFU.

Conclusions: The risk of developing active TB in HIV-infected patients with positive T-Spot.TB receiving combination antiretroviral therapy is low in Taiwan where the national TB program has led to a sustained decrease in TB incidence.

前言

根據過去研究，病患在感染結核病後，通常進入潛伏結核感染狀態，即臨床上無症狀，也無法從病患身上培養出結核菌，但皮膚結核菌素測驗結果為陽性。就一般大眾而言，從潛伏結核感染狀態進展為活動性結核病，一生約僅有10%左右的機會[1]。雖然感染後不易發病，但有些人發病的危險性較高，如愛滋病毒感染者、器官移植者、長期使用類固醇病患、慢性腎衰竭進而洗腎者等。而HIV 病毒感染者若有潛伏結核感染，每年發生活動性結核病的機會約10%[1]。

不論是在罹患HIV病毒感染者或非HIV病毒感染者的族群中，不少研究證實治療潛伏結核感染可有效地減少日後發病的機會 [2, 3]。根據後設分析(meta-analysis)，在11個隨機對照試驗 (randomized controlled trials) 中，包括73,375位非HIV病毒感染者，治療潛伏結核感染後追蹤兩年或更久，其發生活動性結核病之相對危險性(relative risk, RR)為0.40(95%信賴區間，0.31-0.52) [3]; 在12個隨機對照試驗，包括8,578位HIV病毒感染者，治療潛伏結核感染後，其發生活動性結核病之相對危險性為0.68 (95%信賴區間，0.54-0.85)；此保護效果，在皮膚結核菌素測驗陽性者效果更好 (RR 0.38； 95%信賴區間，0.25-0.57) [2]。這些隨機對照試驗亦包含在結核病盛行率較高之地區進行之隨機對照試驗，如非洲國家、菲律賓、巴西、西班牙和香港等[2, 3]。

除了用isoniazid治療潛伏結核感染可有效地減少日後HIV病毒感染者發病的機會外，研究亦證實高效能抗病毒藥物的使用可減少HIV病毒感染者發生結核病，不管其CD4數為和數為何 [4]。有鑒於此，世界衛生組織在2009年建議高效能抗病毒藥物應用於所有HIV病毒感染且CD4數小於350的病患，及所有罹患結核病的HIV病毒感染者，不管其CD4數為和數為何 [5]。而另一研究顯示，在結核病發生率偏高的地域，預防性isoniazid應使用於所有接受高效能抗病毒藥物的HIV病毒感染者，以預防HIV病毒感染者發生活動性結核病[6]。

皮膚結核菌素測驗多年來一直為診斷潛伏結核感染的唯一工具，但其結果會受卡介苗施打或暴露過非結核分枝桿菌影響(non-tuberculosis mycobacteria)。 再者，皮膚結核菌素測驗從施打到判讀需兩次就診 (相隔48-72小時)，在皮膚結核菌素施打後，病患易因其他因素(如工作或假日不看診)，無法在預定時間內回診判讀施打結果。此外，皮膚結核菌素施打結果判讀不易，不同人員判讀結果可能會有差異。最後，在免疫不良的族群中，皮膚結核菌素測驗的敏感性亦受到影響。

有鑒於上述皮膚結核菌素測驗的限制，Interferon Gamma Release Assay (IGRA, 丙型干擾素)為一新發展出診斷潛伏結核感染的檢驗，其檢驗結果不會因卡介苗接種或曾暴露過非結核分枝桿菌而受到影響，且只要抽血一次，就可得到結果。目前IGRA包括：QuantiFERON-TB (QFT)及T-SPOT.TB兩種試劑。QFT根據實驗技術的改善，又再分為QuantiFERON-TG Gold test(QFT-G)及QuantiFERON-TG Gold In-Tube test(QFT-GIT)。

因潛伏結核感染目前並無公認的診斷標準，一後設分析以TB培養陽性病患為診斷標準，T-SPOT.TB診斷的敏感性(sensitivity)(90%, 95%信賴區間86%-93%)優於QFT(78%, 95%信賴區間73%-82% in QFT-Gold; 70%, 95%信賴區間63%-78% in QFT-Gold In-Tbue)及皮膚結核菌素測驗(77%, 95%信賴區間71%-82%)。在施打卡介苗地區，診斷結核感染的特異性T-SPOT.TB(93%, 95%信賴區間86%-100%)及QFT(96%, 95%信賴區間94%-98%)皆優於及皮膚結核菌素測驗(59%, 95%信賴區間46%-73%) [7]。

若以追蹤後來發生活動性結核病為診斷標準, IGRA和皮膚結核菌素測驗相較, 兩者對預測將來發生活動性結核病的能力相當, 但IRGA的檢測可減少偽陽性的狀況發生[8]. 即便如此，IGRA對未來預測HIV病毒感染者發生結核病的研究為數仍少 [9, 10]。

自西元2011年三月至西元2013年九月,在台大醫院共收募617位HIV病毒感染者接受T-Spot檢測; 617位病患中有535位(86.7%)T-SPOT.TB陰性, 72位(11.7%) T-SPOT.TB陽性，10位(1.6%)T-SPOT.TB結果為無法判定(indeterminate)。本研究欲評估 T-SPOT.TB陽性在HIV感染病患的盛行率及危險因子。了解活動性結核病在T-SPOT.TB陽性HIV感染病患的發生率及罹患活動性結核病的危險因子。

材料與方法

納入條件

接受T-Spot檢測且沒有罹患活動性結核病的HIV病毒感染者

追蹤時間

自接受T-Spot檢測時間開始至病患死亡或西元2015年十月三十日為止。

資料收集

1. 每次檢驗 T-SPOT.TB 時病患之 CD4 及病毒量，是否同時有伺機性感染或使用高效能抗病毒藥物。
2. 是否曾罹患結核病。
3. 完整活動性結核病評估結果。
4. 追蹤結束時病患是否發生活動性結核病，及當時的 CD4 及病毒量，及是否有使用高效能抗病毒藥物。

分析方法

研究主持人採用制式的Excel紀錄，並將運用SPSS統計和分析資料。

1. 評估陽性 T-Spot.TB 在 HIV 感染病患的盛行率及危險因子。
2. 了解活動性結核病在陽性全血丙型干擾素 HIV 感染病患的發生率。
3. 了解活動性結核病的危險因子。

結果

自西元2011至2015年十月三十日止，共617位曾檢測過丙型干擾素的HIV病毒感染病患中，排除九位在檢驗當下有活動性肺結核的病患(一位檢驗結果為陰性，八位檢驗結果為陽)，剩下608位檢測當下沒有活動性肺結核的病患納入本研究，81%為男同志，15.7%為異性戀，1.5%為靜脈毒癮使用者，1.8%為其他族群。有534位(87.8%)檢測結果為陰性，64位(10.5%)檢測結果為陽性，10位(1.6%)檢測結果為無法判定。

檢測當下，534位檢測結果為陰性的平均CD4值為441.6 cells/μl，平均病毒量為2.89 $\log_{10}$ copies/mL; 64位檢測結果為陽性病患的平均CD4值為578.4 cells/μl，平均病毒量為2.35 $\log_{10}$ copies/mL，追蹤至2015/10/30止，檢測結果為陽性的病患中，僅一位未接受高效能抗病毒藥物治療。10位檢測結果為無法判定的病患，其平均CD4值為473.5 cells/μl，平均病毒量為2.79 $\log_{10}$ copies/mL。

分析檢測結果陽性的相關因子為年紀較大者(調整後每增加十歲的勝算比為1.172, 95%的信賴區間為1.022-1.344, P=0.023)，過去有結核病病史者(調整後的勝算比為13.412, 95%的信賴區間為6.106-29.460, P<0.001)，及CD4值較高者(調整後每增加50CD4值的勝算比為1.062, 95%的信賴區間為1.017-1.109, P=0.007)。

追蹤至2015/10/30止，僅一位檢測結果陽性患者沒有接受高效能抗病毒藥物治療。另一位檢測結果陽性患者在追蹤337天後發生活動性結核病，在結核病診斷當下，病患已接受雞尾酒治療，當時的CD4為78 cells/uL，HIV病毒量為98 copies/mL。故64位檢測結果為陽性患者的結核病發生率為每100人年0.422件。 534位檢測結果為陰性，在追蹤1990人年後，沒有人發生結核病。24位檢測結果陽性且曾罹患過結核病的患者，在追蹤88人年後，沒有人發生結核病。

結論與建議

在本研究中，接受丙型干擾素檢測的HIV病毒感染病患中，陽性結果的盛行率約為10.5%。檢測陽性的患者中，在追蹤過程中僅一位發生活動性結核病，故陽姓預測率為1.6% (1/64)。而64位檢測結果為陽性患者的結核病發生率為每100人年0.422件。過去文獻中，丙型干擾素檢測陽性的HIV病毒感染病患中，其結核病發生率為每100人年0到4.621件 [9-13]。相較於其他研究，本研究的結核病發生率(每100人年0.422件)算低，會有這些差異主要是因執行研究所在地結核病的發生率不同，納入研究的病患數不一致(從50到909人不等)，追蹤的時間不等(1.28年至2.97年不等)，各研究中診斷偵測結核病的方法不一，再者，有些病患接受潛伏性結核病的治療，有些接受接受高效能抗病毒藥物治療(0-100%)，病患的CD4數(305-447 cells/uL)和HIV病毒數(1.85-4.8 $\log_{10}$ copies/mL)不同所致。

為減少丙型干擾素檢測陽性的HIV病毒感染病患進展到活動性結核病，及早開始高效能抗病毒藥物治療及接受潛伏性結核病的治療為為兩項最主要的方法 [4, 6, 14, 15]。但有不少病患在HIV感染末期才診斷出HIV感染 [16, 17]。需更加強HIV的早期診斷及讓感染病患能留在醫療體系追蹤，已接受高效能抗病毒藥物治療，成功控制HIV病毒 [18]。

本研究族群，81%為男同志，僅1.5%為靜脈毒癮使用者，故本研究結論無法推估到其他不同的HIV感染族群。再者，因僅一位病患進展成活動性結核病，本研究無法再分析丙型干擾素檢測陽性的HIV病毒感染病患進展到活動性結核病的危險因子。此外，追蹤過程中，並不是所有的病患都接受結核病的檢查，而是根據臨床症狀安排結核病的檢查，故可能低估結核病的發生率。最後，隨著年齡的老化和共病的發生，HIV病毒感染病患罹患結核病的風險可能漸漸增加。

最近資料顯示，國內結核病發生率從西元2005年的每十萬人口72位下降至西元2012年的每十萬人口僅53位 [19]。本研究發現在整體結核病發生率下降的台灣社會中，接受高效能抗病毒藥物治療的HIV病毒感染病患發生結核病的風險低。

對政策之具體建議: HIV病毒感染病患應該接受高效能抗病毒藥物治療以降低結核病發生率。

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附件十五

計畫編號：MOHW104-CDC-C-114-000301

衛生福利部疾病管制署 104 年委託科技研究計畫

計畫名稱：接受高效能抗反轉錄病毒治療之後天免疫不全病 毒感染患者合併 C 型肝炎病毒感染中接受長效型干擾素合併雷巴威林後之長期病毒預後研究

年 度 研 究 報 告

執行機構：國立台灣大學醫學院附設醫院

計畫主持人：劉振驊

執行期間： 104 年 1 月 1 日至 104 年 10 月 31 日

＊本研究報告僅供參考，不代表本署意見，如對媒體發布研究成果應事先徵求本署同意*

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摘要 (中文)

亞太地區相關愛滋病毒感染者並有慢性或急性 C 型肝炎接受 peginterferon 和 ribavirin 的資料非常有限。在台大醫院，我們針對並有愛滋病毒和 C 型肝炎病毒共同感染的病患給予 24 週 peginterferon 依照體重調整劑量的(24 位急性 HCV 感染者)，針對慢性感染基因型第一或第六型(HCV 1/6)。依據前四週和第十二週的治療效果給予 24-72 週或者基因型第二型或者第三型 (HCV-2/3) 24-48 週的治療 (慢性感染共 92 位)。我們發現在停藥後 24 周依然沒有能檢測到 C 型肝炎病毒的機會在急性和慢性感染病患分別是 83%與 72% (p=0.30); 如果是感染基因型第一或第六型(HCV 1/6)的療效是 68%，而基因型第二或者第三型(HCV-2/3)的療效是 72%。我們同時發現年紀(odds ratio [OR] per 1-year increase: 0.88, 95% confidence interval [CI]: 0.78-0.99, p=0.04)、HCV 病毒量(OR per 1-$\log_{10}$ increase: 0.18, 95% CI: 0.03-0.98, p=0.03)、 *IL28B* 基因型 (OR: 5.52, 95% CI: 1.55-12.2, p=0.02)和在第四週即能達到病毒檢測不到 (OR: 9.62, 95% CI: 3.89-15.3, p=0.007) 能預測基因型第一或第六型的慢性HCV感染者能達到停藥24週後檢測不到病毒的機會。

關鍵字: C 型肝炎病毒, 愛滋病毒感染, 長效型干擾素, 雷巴威靈, 亞洲人

摘要 (英文)

Data are limited on the effectiveness of peginterferon plus ribavirin in HIV-infected Asian patients with acute or chronic HCV infection. HIV-infected Taiwanese patients with acute HCV infection received peginterferon plus weight-based ribavirin for 24 weeks (n=24), and those with chronic HCV genotype 1 or 6 (HCV-1/6) and HCV genotype 2 or 3 (HCV-2/3) infection received response-guided therapy for 24-72 and 24-48 weeks, respectively (n=92). The primary endpoint was sustained virologic response (SVR), defined as undetectable HCV RNA 24 weeks off-therapy. The SVR rates were 83% and 72% in patients with acute and chronic HCV infection (p=0.30), and 68% and 72% in patients with chronic HCV-1/6 and HCV-2/3 infection (p=0.48), respectively. While no factors predicted SVR in acute HCV and chronic HCV-2/3 infection, age (odds ratio [OR] per 1-year increase: 0.88, 95% confidence interval [CI]: 0.78-0.99, p=0.04), HCV viral load (OR per 1-$\log_{10}$ increase: 0.18, 95% CI: 0.03-0.98, p=0.03), *IL28B* genotype (OR: 5.52, 95% CI: 1.55-12.2, p=0.02), and RVR (OR: 9.62, 95% CI: 3.89-15.3, p=0.007) predicted SVR in chronic HCV-1/6 infection. In conclusion, the SVR rates of peginterferon plus ribavirin for 24 weeks and for response-guided 24-72 weeks, respectively, are satisfactory in HIV-infected Taiwanese patients with acute and chronic HCV infection.

Key words: hepatitis C virus, human immunodeficiency virus, peginterferon, ribavirin, Asian

一、前言

因為有共同的感染危險因子所以愛滋病毒感染者也容易併有C型肝炎病毒感染根據估計全球大約有一千萬人併有愛滋病毒和C型肝炎病毒感染。[1] 愛滋病毒感染者如果併發慢性C型肝炎感染較容易會發生慢性肝病的併發症。[2,3] 同時不管病患是否同時接受抗愛滋病毒藥物治療血液中C型肝炎病毒量較高、較容易發生母嬰間垂直傳染、肝臟纖維化的速度較快、肝臟相關的疾病或死亡率都會增加。[4-8] 反之如果能夠接受有效的抗C型肝炎藥物並且清除病毒預後可以獲得大幅改善。[9,10]

愛滋病毒感染 C 型肝炎病毒的治療在過去也是使用長效型干擾素和雷巴威靈。急性 C 型肝炎的病患在這樣的治療下達到停止治療後 24 周依然檢測不到 C 型肝炎病毒的機會大約是 60-80%。[11] 但是對於慢性 C 型肝炎相同的治療使用 24-48 周的治療療效大約僅有 27-50%。[12-16] 再者感染基因型第一型或者第四型的療效遠比感染第二型或者第三型來得差 (14-38% versus 44-73%)。即便是能夠在第四週就達到檢測不到 C 肝病毒的病患可以縮短治療時間但是真正能達到第四週的療效的比例不論是哪一種基因型機會都很低 (18-32% versus 65%, HCV-1/4 and HCV-2/3 infection)。[17,18] 對於高 C 型肝炎病毒量的患者 (> 400,000-800,000 IU/mL)普遍治療效果都很差。[12,18-20]

目前國際間已經有直接抑制病毒複製的藥物(direct-acting antiviral agents (DAAs))。有研究顯示針對急性 C 型肝炎病毒感染的愛滋病毒感染者給予 telaprevir 併用長效型干擾素和雷巴威靈能夠清除 C 型肝炎病毒的機會達到 84%[21] ，而接受 boceprevir or telaprevir 併用長效型干擾素和雷巴威靈能夠清除 C 型肝炎病毒的機會大約是 63-74%[2,3] 。目前最新的研究都顯示使用 12 到 24 週的新型抗 C 型肝炎藥物的療效大約 76-97%。[24-27] 雖然如此，這些藥物組合非常昂貴，在資源較缺乏的國家或地區鮮少有人能負擔得起這樣的新型藥物治療。

亞洲地區關於愛滋病毒感染者的 C 型肝炎病毒治療經驗鮮少有學者報告。在日本有兩個小型的研究顯示對於急性和慢性 C 型肝炎感染的治療療效大約是 75%和 60%。[28,29] 而且有一病患在接受含有 telaprevir 的治療有效地清除 C 型肝炎病毒[30] 。雖然這些新型藥物在亞洲普及率依然很低，亞洲人有較好的基因型 interleukin-28B (*IL28B*) interleukin-28B (*IL28B*)對於長效型干擾素和雷巴威靈的效果較白人好。因此本研究就是想針對愛滋病毒感染的國人同時併有急性或者慢性 C 型肝炎病毒感染接受效型干擾素和雷巴威靈的效果。

二、材料與方法

1. 受試者納入條件

所有於西元2009-2014年間在台大醫院就醫的愛滋病感染者併有急性或者慢性C型肝炎病毒感染，同時血中偵測到C型肝炎病毒。病患如果有腎功能不全（serum creatinine level ≥ 1.5 times ULN)、已經代償不全的肝硬化、精神疾患、骨髓功能不佳（hemoglobin levels < 13 g/dL for men or < 12 g/dL for women; neutrophil count < 1.5 x 10^9 cells/L; platelet count < 90 x 10^9 cells/L; CD4 count < 300 x 10^6 cells/L）、懷孕、控制不良的免疫相關疾病、併發視網膜、併發第二型糖尿病、心肺病變等病人，並不適合接受干擾素和雷巴威靈的治療。針對急性 C 型肝炎病毒感染的患者，必須觀察十二週後，血液中依然偵測得到 C 型肝炎病毒，才能納入治療的研究。

2. 治療處方含有 alfa-2a 180 μg/week (Pegasys, Hoffman-LaRoche, Basel, Switzerland) 加上依據體重調整劑量的雷巴威靈 (Copegus, Hoffman-LaRoche, Basel, Switzerland; 體重低於 75 公斤的病患使用 1,000 mg/day，對於體重超過 75 公斤的病患使用 1,200 mg/day)。治療的療程決定則是依照基因型和治療反應決定。

3. 急性 C 型肝炎病毒感染，治療期程為 24 週。感染 HCV-1/6 的慢性 C 型肝炎感染者如果病毒量較低(低於800,000 IU/mL)而且能在四週治療後即檢測不到C型肝炎病毒，治療療程為 24 週; 如果屬於高病毒量而且能在四週或八週治療後檢測不到病毒，療程是 48 週。如果病患無法在第八週達成檢測不到病毒，但是到第十二週時病毒量下降超過一百倍，那麼療程必須延長到 72 週 (Arms A-D) (圖一)。

針對基因型 HCV-2/3，原則上如果能在第四週達成病毒量檢測不到就治療 24 週；否則就治療 48 週 (Arms F and G)。治療到第十二週時病毒量依然無法下降達一百倍以上，就不再延長治療 (Arms E and H)。

4. 我們收集病患的基礎和追蹤的相關愛滋病毒治療資料,其他慢性肝炎、生化檢驗結果、以及 interleukin-28B (*IL28B*)基因型，以評估藥物副作用和療效。C 型肝炎病毒量在治療開始的第四週、第八週、第十二週和往後每十二週檢測，一直到治療結束後持續追蹤 24 週，已確認達到 C 型肝炎病毒清除而且沒有復發。

5. 資料之蒐集處理評估

研究主持人和協同主持人採用制式的 Excel 紀錄病人資料。我們已描述性統計方式記述病患的基礎資料和療效。同時我們嘗試以多變項的統計方式嘗試找尋影響接受治療者在完成治療後追蹤 24 週時能達成依然檢測不到病毒量的影響因子。

三、結果

1. 收案的流程如圖一所顯示。最終納入治療分析的人數，急性和慢性C型肝炎病毒感染的人數分別是24和92人。
2. 收納接受治療和分析的病患的基礎點相關臨床資料，如表一所示。
3. 依據原先設定的急性或者慢性C型肝炎病毒感染的治療療程， 如圖二所示。
4. 急性和慢性C型肝炎病毒感染的愛滋病毒感染患者開始接受長效型干擾素和雷巴威靈後在第四週、第八週和第十二週時，血液中病毒量檢測不到的比例，如表二所顯示。急性C型肝炎病毒感染者，全數都在第四週達到病毒量檢測不到，但是慢性C型肝炎病毒感染者僅有54%達到(P<0.001) 。治療到第十二週時，病毒量檢測不到的比例，在兩組分別是100%和96% (P=0.53)。而在完治後追蹤24週時病毒量檢測不到的比例，在兩組分別是83%和72% (P=0.3)
5. 基因型別和第四週、第八週、第十二週，以及在完治後追蹤24週時病毒量檢測不到的比例，分別顯示在表三中。
6. 相關接受長效型干擾素和雷巴威靈過程中的副作用發生比例，如表四所顯示。
7. 相關影響在治療結束後續追蹤24週能持續達到病毒量檢測不到的因子，在多變項分析的結果，如表五所顯示。我們發現急性感染者，因為收納人數較少，我們無法找出可以達到統計學上有意義差異的因子。但是針對慢性感染者我們發現年紀 (odds ratio [OR] per-1-year increase: 0.91, 95% confidence interval [CI]: 0.81-0.99, p=0.03)、高病毒量(OR per 1-$\log_{10}$ increase: 0.35, 95% CI: 0.14-0.84, p=0.02)、*IL28B* 基因型(OR: 5.56, 95% CI: 2.39-8.33, p=0.006)、和能在第四週即可達成病毒量檢測不到 (OR: 8.33, 95% CI: 5.89-14.3, p=0.003) 都是能預測在治療結束後續追蹤24週能持續達到病毒量檢測不到的因子。
8. 不同基因型感染治療前能夠具有預測療效的因子, 如表六所顯示。針對感染基因型第一或者第六型的慢性感染, 我們在多變項的分析發現具有預測的因子包含年紀 (OR per 1-year increase: 0.88, 95% CI: 0.78-0.99, p=0.04)、高病毒量 (OR per 1-$\log_{10}$ increase: 0.18, 95% CI: 0.03-0.98, p=0.03)、*IL28B* 基因型(OR: 5.52, 95% CI: 1.55-12.2, p=0.02 和能在第四週即可達成病毒量檢測不到(OR: 9.62, 95% CI: 3.89-15.3, p=0.007) 。

四、結論與建議

在這個觀察研究中，我們發現台灣的愛滋病毒感染者併有慢性或者急性的C型肝炎病毒感染時，給予長效型干擾素和雷巴威靈，分別有72%和83%的病患能夠在治療結束後續追蹤24週能持續達到C型肝炎病毒量檢測不到。這個治療成果相較西方國家的雙重病毒感染者也接受相同的治療處方來得好。因為藥物高價位的因素，目前國內能夠接受新的directly-acting antivirals的病患人數勢必很少。絕大多數依然需要接受傳統的治療方式 因此本研究可以提供國內臨床醫師和健保署相關單位參考。其次，因為急性C型肝炎病毒感染有較高的機會可以在接受傳統藥物治療即可治癒，臨床醫療人員在遇見病患出現肝功能異常，尤其又併有像梅毒的性病發生時應儘快提供C型肝炎抗體檢測和追蹤，以期能早點診斷急性C型肝炎病毒感染和轉介接受適當的治療，以降低繼續傳染他人的風險。

五、重要研究成果及具體建議

近期國內愛滋病毒感染男同志的C型肝炎病毒感染的發生率持續增加。除了行為介入以外，提供定期的追蹤檢測和適當有效的治療，在有效的疫苗開發應用之前，是防治C型肝炎擴散傳染的最有效方法。

對政策之具體建議:對於國內男同志提供定期的HCV追蹤檢測和適當有效的治療，是防治C型肝炎擴散傳染的最有效方法。

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Table 1. Patient characteristics

Variables*	All patients (N = 116)			Chronic HCV infection (N = 92)		
	Acute HCV infection (N = 24)	Chronic HCV infection (N = 92)	*p* value	Genotype 1/6 (n = 53)	Genotype 2/3 (n = 39)	*p* value
Mean age (SD), y	35 (8)	38 (8)	0.15	38 (8)	37 (8)	0.48
Sex			0.99			0.99
Male	22 (92)	84 (91)		48 (91)	36 (92)	
Female	2 (8)	8 (9)		5 (9)	3 (8)	
Mean hemoglobin level (SD), g/dL	15.6 (1.3)	15.1 (1.3)	0.18	14.8 (1.3)	15.4 (1.2)	0.12
Mean white blood cell count (SD), 10^6 cells/L	5,555 (1,456)	6,064 (1,732)	0.19	6,148 (1,748)	5,951 (1,728)	0.59
Mean platelet count (SD), 10^9 cells/L	239 (63)	210 (68)	0.06	211 (64)	208 (74)	0.83
Mean albumin level (SD), g/L	4.7 (0.2)	4.6 (0.4)	0.60	4.5 (0.4)	4.6 (0.4)	0.30
Mean total bilirubin level (SD), mg/dL	1.2 (0.6)	1.2 (0.9)	0.88	1.1 (0.8)	1.2 (1.0)	0.47
Mean ALT quotient (SD)	7.3 (6.7)	3.3 (3.2)	< 0.001	3.2 (3.0)	3.4 (3.1)	0.27
HIV risk behavior			0.06			0.03
IDU	2 (8)	27 (29)		20 (38)	7 (18)	
MSM	22 (92)	62 (67)		30 (57)	32 (82)	
Hemophilia	0 (0)	3 (3)		3 (6)	0 (0)	
On HAART	23 (96)	83 (90)	0.69	49 (92)	34 (87)	0.49
Mean HIV RNA (SD), copies/mL	5,550 (26,934)	3,972 (15,294)	0.71	3,976 (15,137)	3,967 (15,704)	0.99
HIV-RNA < 50 copies/mL	18 (75)	58 (63)	0.34	34 (64)	24 (62)	0.83
Mean CD4 cell count (SD), 10^6 cells/L	439 (92)	428 (85)	0.21	415 (72)	445 (99)	0.10
HCV RNA (SD), $\log_{10}$ IU/mL	5.00 (1.45)	6.16 (1.36)	< 0.001	6.36 (1.16)	5.90 (1.60)	0.11

HCV RNA ≥ 800,000 IU/mL	8 (33)	64 (70)	0.002	38 (72)	26 (67)	0.65
HCV genotype			0.61			< 0.001
1	10 (42)	37 (40)		37 (70)	0 (0)	
1a	1 (4)	9 (10)		9 (17)	0 (0)	
1b	9 (38)	28 (30)		28 (53)	0 (0)	
2	12 (50)	34 (37)		0 (0)	34 (87)	
2a	12 (50)	30 (33)		0 (0)	30 (77)	
2b	0 (0)	4 (4)		0 (0)	4 (10)	
3a	0 (0)	4 (4)		0 (0)	4 (10)	
6a	1 (4)	8 (9)		8 (15)	0 (0)	
Mixed†	1 (4)	9 (10)		8 (15)	1 (3)	
1a+1b	1 (4)	2 (2)		2 (4)	0 (0)	
1+2	0 (0)	4 (4)		4 (8)	0 (0)	
1+6a	0 (0)	2 (2)		2 (4)	0 (0)	
2+3a	0 (0)	1 (1)		0 (0)	1 (3)	
***IL28B* rs8099917 genotype**			0.80			0.45
TT	19 (79)	67 (73)		36 (68)	31 (79)	
GT	4 (17)	21 (23)		14 (26)	7 (18)	
GG	1 (4)	4 (4)		3 (6)	1 (3)	
METAVIR fibrosis stage‡			0.005			0.47
F0	15 (63)	22 (24)		11 (21)	11 (28)	
F1	6 (25)	26 (28)		15 (28)	11 (28)	
F2	2 (8)	22 (24)		15 (28)	7 (18)	
F3	1 (4)	13 (14)		9 (17)	4 (10)	
F4	0 (0)	8 (9)		3 (6)	5 (13)	
Undetermined	0 (0)	1 (1)		0 (0)	1 (3)	

SD, standard deviation; ALT, alanine aminotransferase; HIV, human immunodeficiency virus; IDU, injection drug user; MSM, men who have sex with men; HAART, highly active antiretroviral therapy; CD, cluster of differentiation; RNA, ribonucleic acid; HCV, hepatitis C virus; *IL28B*, interleukin-28B.

* Values are numbers (percentages) unless otherwise indicated.

† Patients with mixed genotypes 1/2, or 1/6a infections were treated by the same protocol as those with genotype 1/6 monoinfection.

‡ The stage of fibrosis was assessed by transient elastography (Fibroscan®, Echosens, Paris), and was determined according to the cut-off values proposed by Castera, et al.[32]

Table 2. Virologic responses in HIV-infected patients with acute or chronic HCV infection

Virologic response*	Acute HCV infection (N = 24)	Chronic HCV infection (N = 92)	*p* value
RVR	24 (100)	50 (54)	< 0.001
EVR	24 (100)	88 (96)	0.58
ETVR	23 (96)	86 (93)	0.99
SVR	20 (83)	66 (72)	0.30
Non-SVR	4 (17)	26 (27)	
Relapse	2 (8)	15 (16)	
No response	0 (0)	4 (4)	
Breakthrough	1 (4)	2 (2)	
Undetermined	1 (4)	5 (5)	

HIV, human immunodeficiency virus; HCV, hepatitis C virus; RVR, rapid virologic response; EVR, early virologic response; ETVR, end-of-treatment virologic response; SVR, sustained virologic response.

* Values are numbers (percentages).

Table 3. Virologic responses in HIV-infected patients with chronic HCV genotype 1/6 or genotype 2/3 infection

Virologic response*	Genotype 1/6 (n = 53)						Genotype 2/3 (n = 39)				p value†
	Arm A (n =12)	Arm B (n =10)	Arm C (n = 16)	Arm D (n = 11)	Arm E (n = 4)	All (A-E) (n = 53)	Arm F (n = 28)	Arm G (n = 11)	Arm H (n = 0)	All (F-H) (n = 39)	
RVR	12 (100)	10 (100)	0 (0)	0 (0)	0 (0)	22 (41)	28 (100)	0 (0)	-	28 (72)	0.006
Wk-8R	-	-	16 (100)	0 (0)	0 (0)	-	-	-	-	-	-
EVR	12 (100)	10 (100)	16 (100)	11 (100)	0 (0)	49 (92)	28 (100)	11 (100)	-	39 (100)	0.13
ETVR	12 (100)	10 (100)	15 (94)	10 (91)	0 (0)	47 (89)	28 (100)	11 (100)	-	39 (100)	0.04
SVR	11 (92)	9 (90)	12 (75)	4 (36)	0 (0)	36 (68)	23 (82)	7 (64)	-	30 (77)	0.48
Non-SVR	1 (8)	1 (10)	4 (25)	7 (64)	4 (100)	17 (32)	3 (11)	6 (55)	-	9 (23)	
Relapse	1 (8)	1 (10)	2 (13)	5 (45)	0 (0)	9 (17)	2 (7)	4 (36)	-	6 (15)	
No response	0 (0)	0 (0)	0 (0)	0 (0)	4 (100)	4 (8)	0 (0)	0 (0)	-	0 (0)	
Breakthrough	0 (0)	0 (0)	1 (6)	1 (9)	0 (0)	2 (4)	0 (0)	0 (0)	-	0 (0)	
Undetermined	0 (0)	0 (0)	1 (6)	1 (9)	0 (0)	2 (4)	1 (4)	2 (18)	-	3 (8)	

HIV, human immunodeficiency virus; HCV, hepatitis C virus; RVR, rapid virologic response; Wk-8R, week 8 virologic response; EVR, early virologic response; ETVR, end-of-treatment virologic response; SVR, sustained virologic response.

* Values are numbers (percentages).

† Comparison for patients with HCV genotype 1/6 and HCV genotype 2/3 infections.

Table 4. Adverse events in HIV-infected patients with acute or chronic HCV infection

Parameter*	Acute HCV infection	Chronic HCV infection						
	All (N = 24)	Arm A (N =12)	Arm B (N =10)	Arm C (N = 16)	Arm D (N = 11)	Arm E (N = 4)	Arm F (N = 28)	Arm G (N = 11)
Flu-like symptoms	7 (29)	4 (33)	3 (30)	4 (25)	4 (36)	2 (50)	9 (32)	3 (27)
Fatigue	6 (25)	4 (33)	4 (40)	6 (38)	2 (22)	1 (25)	8 (29)	2 (22)
Headache	2 (8)	1 (8)	1 (10)	1 (6)	1 (9)	0 (0)	3 (11)	1 (9)
Insomnia	6 (25)	4 (33)	3 (30)	4 (25)	3 (27)	1 (25)	9 (32)	2 (18)
Irritability	1 (4)	1 (8)	1 (10)	2 (13)	1 (9)	0 (0)	3 (11)	1 (9)
Depression	1 (4)	0 (0)	1 (10)	1 (6)	0 (0)	0 (0)	2 (7)	0 (0)
Anorexia	4 (17)	2 (17)	1 (10)	2 (13)	2 (22)	1 (25)	5 (18)	2 (18)
Diarrhea	1 (4)	0 (0)	0 (0)	1 (6)	1 (9)	0 (0)	2 (7)	0 (0)
Cough	1 (4)	0 (0)	0 (0)	1 (6)	1 (9)	0 (0)	2 (7)	0 (0)
Oral ulcer	2 (8)	1 (8)	1 (10)	1 (6)	1 (9)	1 (25)	3 (11)	1 (9)
Dermatitis	3 (13)	3 (25)	3 (30)	3 (19)	2 (22)	1 (25)	7 (25)	3 (27)
Injection site reaction	1 (4)	0 (0)	0 (0)	1 (6)	0 (0)	0 (0)	1 (4)	1 (9)
Hair loss/alopecia	2 (8)	1 (8)	1 (10)	3 (19)	2 (22)	0 (0)	3 (11)	2 (22)
Anemia†	3 (13)	2 (17)	3 (30)	4 (25)	4 (36)	0 (0)	5 (18)	3 (27)
Leukopenia‡	2 (8)	1 (8)	2 (20)	3 (19)	3 (27)	0 (0)	3 (11)	2 (22)
Thrombocytopenia§	0 (0)	0 (0)	0 (0)	1 (6)	0 (0)	0 (0)	2 (7)	0 (0)

* Values are numbers (percentages).

† Anemia was defined as a nadir hemoglobin level < 10.0 g/dL.

‡ Leukopenia was defined as a nadir absolute neutrophil count (ANC) < 1,500 * 10^6 cells/L

§ Thrombocytopenia was defined as a nadir platelet count < 50 * 10^9 cells/L.

Table 5. Factors predictive of SVR in HIV-infected patients with acute or chronic HCV infection

Variables*	Acute HCV infection (N = 24)					Chronic HCV infection (N = 92)				
	Univariate analysis			Multivariate analysis†		Univariate analysis			Multivariate analysis†	
	SVR (n = 20)	Non-SVR (n = 4)	*p* value	OR (95% CI)	*p* value	SVR (n = 66)	Non-SVR (n = 26)	*p* value	OR (95% CI)	*p* value
Mean age (SD), y	33 (5)	41 (15)	0.05	0.85 (0.73-1.01)	0.07	36 (7)	41 (10)	0.01	0.91 (0.81-0.99)	0.03
Sex (male vs. female)	19/1 (95/5)	3/1 (75/25)	0.31	-	-	61/5 (92/8)	23/3 (88/12)	0.68	-	-
Mean ALT quotient (SD)	7.4 (6.6)	6.9 (8.6)	0.89	-	-	3.2 (3.3)	3.7 (3.1)	0.50	-	-
HIV risk behavior (MSM vs. non-MSM)	19/1 (95/5)	3/1 (75/25)	0.31	-	-	48/18 (73/27)	14/12 (54/46)	0.09	0.93 (0.21-4.06)	0.92
On HAART	20 (100)	3 (75)	0.17	-	-	60 (91)	23 (88)	0.71	-	-
HIV-RNA < 50 copies/mL	16 (80)	2 (50)	0.25	-	-	47 (71)	11 (42)	0.02	2.13 (0.86-4.13)	0.28
Mean CD4 cell count (SD), 10^6 cells/L	500 (102)	461 (125)	0.51	-	-	443 (87)	389 (68)	0.006	1.01 (0.99-1.02)	0.17
HCV RNA (SD), $\log_{10}$ IU/mL	4.75 (1.44)	6.16 (0.86)	0.07	0.17 (0.02-1.64)	0.13	5.93 (1.48)	6.76 (0.75)	0.008	0.35 (0.14-0.84)	0.02
Predominant HCV genotype (1/6 vs. non-1/6) ‡	9/11 (45/55)	3/1 (75/25)	0.59	-	-	36/30 (55/45)	17/9 (65/35)	0.48	-	-
***IL28B* rs8099917 genotype (TT vs. non-TT)**	17/3 (85/15)	2/4 (50/50)	0.18	-	-	54/12 (82/18)	13/13 (50/50)	0.004	5.56 (2.39-8.33)	0.006
METAVIR fibrosis stage (< F2 v.s. ≥ F2)¶	18/2 (90/10)	3/1 (75/25)	0.44	-	-	37/28 (57/43)	11/15 (42/58)	0.25	-	-
RVR (yes vs. no)	20/0 (100/0)	4/4 (100/0)	-	-	-	43/23 (67/23)	7/19 (27/73)	0.006	8.33 (5.89-14.3)	0.003

SD, standard deviation; ALT, alanine aminotransferase; HIV, human immunodeficiency virus; MSM, men who have sex with men; HAART, highly active antiretroviral therapy; CD, cluster of differentiation; RNA, ribonucleic acid; HCV, hepatitis C virus; *IL28B*, interleukin-28B; RVR, rapid virologic response; OR, odds ratio; CI, confidence interval.

* Values are numbers (percentages) unless otherwise indicated.

† Factors with a *p* value < 0.10 in univariate analysis entered multivariate analysis.

‡ Patients with genotype 1 or 6 monoinfection and those with genotypes 1/2, or 1/6a mixed infection were categorized as predominant HCV genotype 1/6 infections.

¶ The transient elastography (Fibroscan®, Echosens, Paris) failed to assess the stage of hepatic fibrosis in one chronic HCV-infected patient who achieved SVR after treatment.

Table 6. Factors predictive of SVR in HIV-infected patients with chronic HCV-1/6 or HCV-2/3 infection

Variables*	HCV-1/6 infection (N = 53)					HCV-2/3 infection (N = 39)				
	Univariate analysis			Multivariate analysis†		Univariate analysis			Multivariate analysis†	
	SVR (n = 36)	Non-SVR (n = 17)	*p* value	OR (95% CI)	*p* value	SVR (n = 30)	Non-SVR (n = 9)	*p* value	OR (95% CI)	*p* value
Mean age (SD), y	37(8)	45 (10)	0.005	0.88 (0.78-0.99)	0.04	35 (7)	35 (9)	0.95	-	-
Sex (male vs. female)	34/2 (94/6)	14/3 (82/18)	0.31	-	-	27/3 (90/10)	9/0 (100/0)	0.99	-	-
Mean ALT quotient (SD)	3.0 (3.5)	1.9 (1.1)	0.18	-	-	3.3 (2.7)	3.2 (3.3)	0.47	-	-
HIV risk behavior (MSM vs. non-MSM)	23/13 (64/36)	7/10 (41/59)	0.15	-	-	25/5 (83/17)	7/2 (78/22)	0.65	-	-
On HAART	34 (94)	15 (88)	0.59	-	-	26 (87)	8 (89)	0.99	-	-
HIV-RNA < 50 copies/mL	27 (75)	7 (41)	0.30	-	-	20 (67)	4 (44)	0.27	-	-
Mean CD4 cell count (SD), 10^6 cells/L	427 (70)	389 (71)	0.08	1.01 (0.99-1.02)	0.38	462 (101)	388(65)	0.05	1.01 (0.99-1.03)	0.06
HCV RNA (SD), $\log_{10}$ IU/mL	6.06 (1.25)	6.99 (0.58)	0.006	0.18 (0.03-0.98)	0.03	5.78 (1.72)	6.33 (0.86)	0.35	-	-
***IL28B* rs8099917 genotype (TT vs. non-TT)**	28/8 (78/22)	8/9 (47/53)	0.03	5.52 (1.55-12.2)	0.02	26/4 (87/13)	5/4 (56/46)	0.07	2.78 (0.86-4.23)	0.07
METAVIR fibrosis stage (< F2 v.s. ≥ F2)¶	20/16 (56/44)	6/11 (35/65)	0.24	-	-	17/12 (59/41)	5/4 (56/44)	0.73	-	-
RVR (yes vs. no)	20/16 (56/44)	2/15 (12/88)	0.003	9.62 (3.89-15.3)	0.007	23/7 (77/23)	5/4 (56/44)	0.24	-	-

SD, standard deviation; ALT, alanine aminotransferase; HIV, human immunodeficiency virus; MSM, men who have sex with men; HAART, highly active antiretroviral therapy; CD, cluster of differentiation; RNA, ribonucleic acid; HCV, hepatitis C virus; *IL28B*, interleukin-28B; RVR, rapid virologic response; OR, odds ratio; CI, confidence interval.

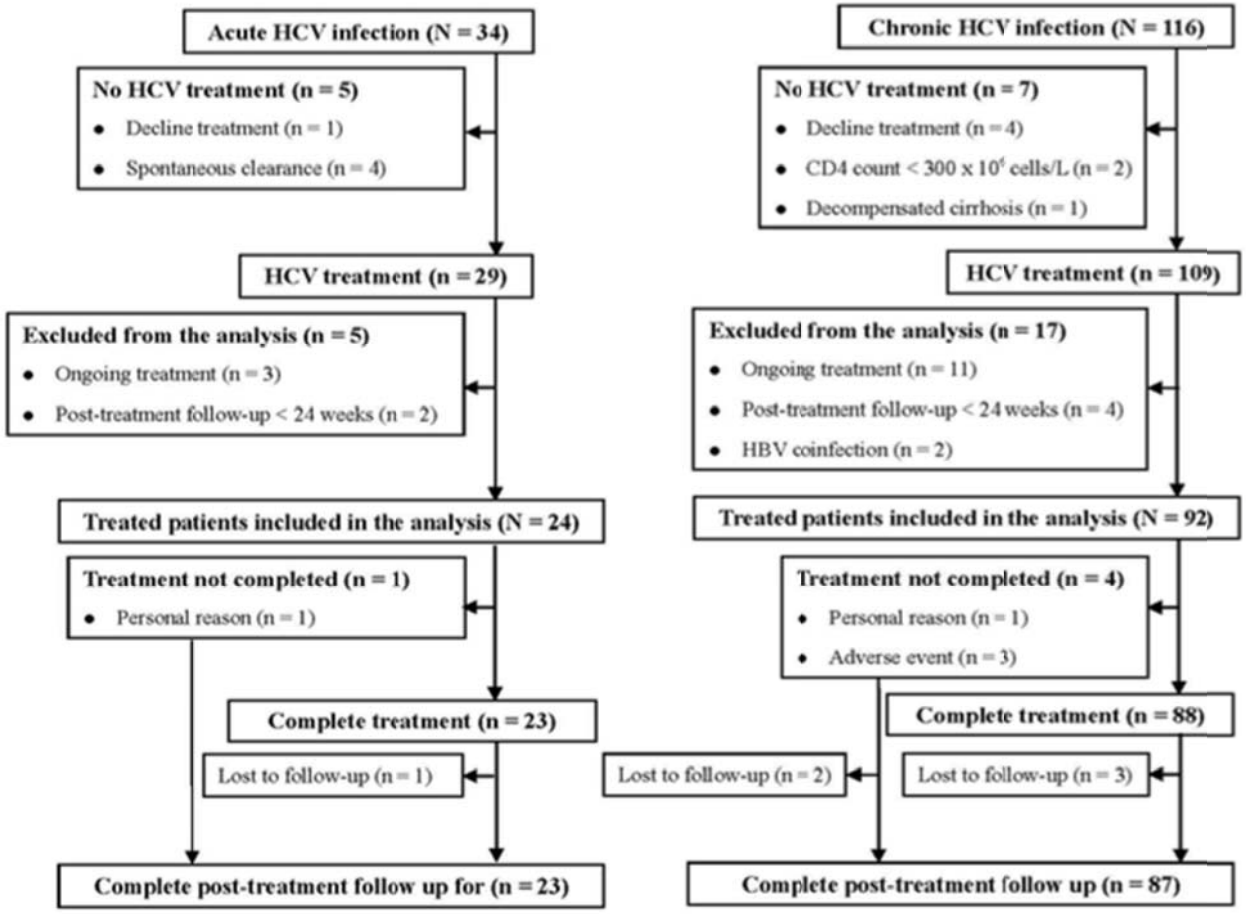
* Values are numbers (percentages) unless otherwise indicated.

† Factors with a *p* value < 0.10 by univariate analysis entered multivariate analysis.

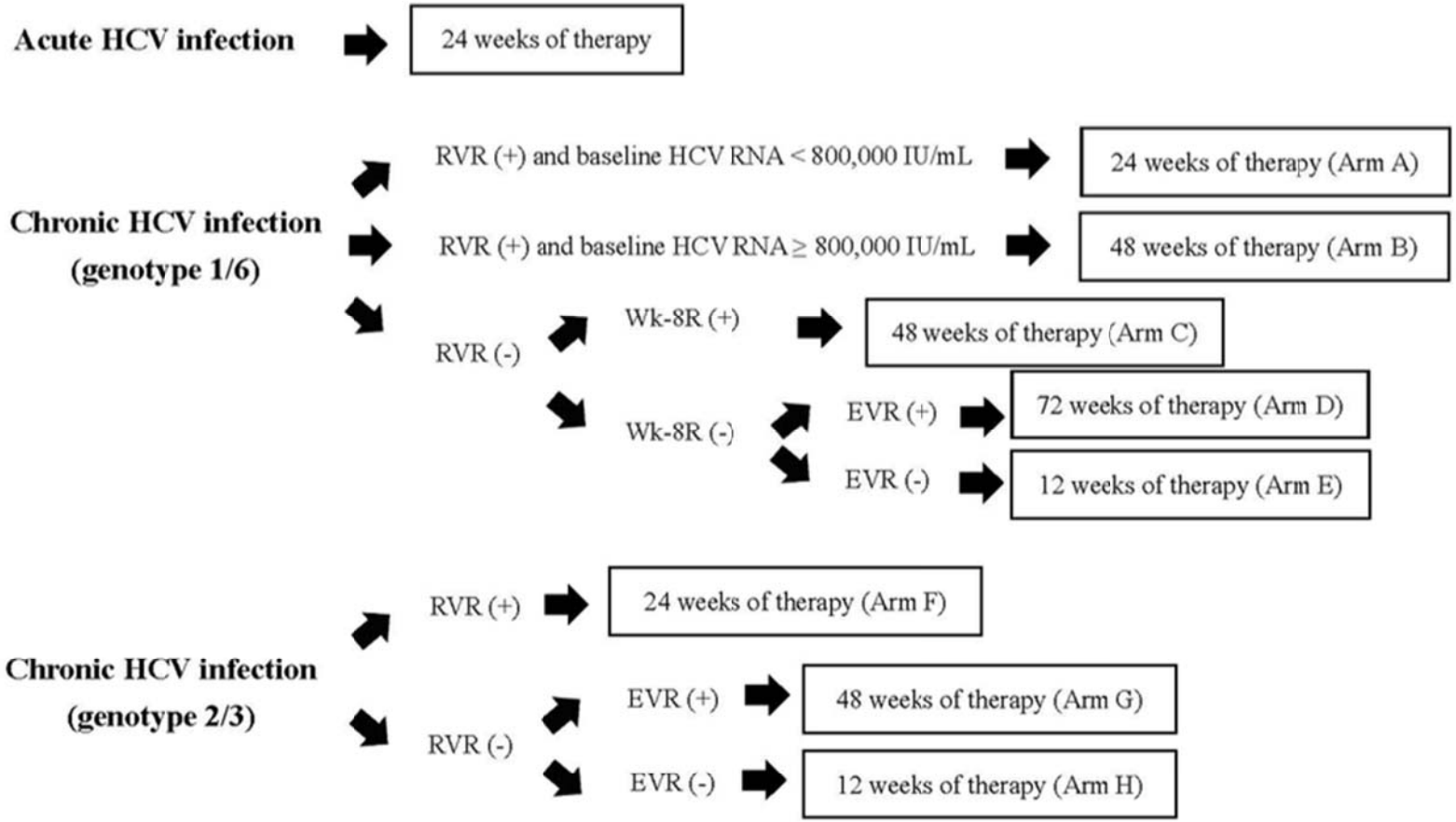
‡ Patients with genotype 1 or 6 monoinfection and those with genotypes 1/2, or 1/6a mixed infection were categorized as predominant HCV genotype 1/6 infections.

¶ The transient elastography (Fibroscan®, Echosens, Paris) failed to assess the stage of hepatic fibrosis in one chronic HCV-2/3 patient who achieved SVR after treatment.

圖一，病患收納接受治療的流程圖



圖二, 病患接受治療後治療期程的流程圖



附　錄

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CORRESPONDENCE

Prevalence of HIV infection and risky sexual practices among adult males with travel-associated *Entamoeba histolytica* infection in Taiwan, 2006–2013

KEYWORDS
Amebiasis;
Entamoeba histolytica;
Sexually transmitted disease;
Sexual behavior;
HIV infection;
Travel medicine;
Taiwan

Dear Editor,

We read with interest the article by Leung et al. [1] that demonstrated that 44% of the laboratory-confirmed cases of *Entamoeba histolytica* infection (EHI) in Taiwan during 2002–2010 were imported from endemic countries, and the incidence of EHI among immigrants/foreign workers was significantly higher than that among Taiwanese who had not traveled aboard. Although epidemiologic features of EHI cases imported through immigration and travel were adequately described in this article, characteristics and associated factors of indigenous EHI cases were not addressed. We have recently described the domestic epidemiology of EHI among Taiwanese adult males during 2006–2013 [2]. HIV-infected males (87% being men who have sex with men [MSM]) have predominated over institutionalized individuals as the major risk groups for indigenous EHI since 2010 and *E. histolytica*-infected males carried a high incidence of subsequent HIV diagnoses, predominantly affecting urban younger MSM. The results suggest a changing domestic epidemiology of male EHI spread associated with sexual behaviors including oral–anal contact [3,4].

To study the interplay of travel and sexual transmission among *E. histolytica*-infected males, we conducted a subgroup analysis of 524 cases of non-institutionalized adult males of Taiwanese nationality with laboratory-confirmed EHI reported through the Notifiable Disease Surveillance Systems during 2006–2013. Eighty-one (15%) males reported recent travel to endemic areas, including mainland China (n = 41), Thailand (n = 15), Vietnam (n = 5), Cambodia (n = 4), Indonesia (n = 4), Malaysia (n = 3), the Philippines (n = 3), India (n = 2), Myanmar (n = 2), Fiji (n = 1), and Palau (n = 1). Of these 81 males, HIV infection and syphilis had been reported in 24 (30%) and 12 (15%) males before EHI diagnosis, respectively. Thirteen males (16%) reported engagement in oral, anal, or oral–anal sex before illness onset of EHI. Of 57 males without a prevalent HIV diagnosis, 12 (21%) males received an HIV diagnosis 3–1389 days (median, 8 days) after EHI diagnosis. In bivariate analysis, *E. histolytica*-infected male travelers who had engaged in oral, anal, or oral–anal sex did not differ significantly from those who had not in terms of age, residing areas, countries of recent travel, and previous syphilis. Engagement in oral, anal, or oral–anal sex before illness onset of EHI was more commonly reported among males with recent travel to endemic areas who had received a HIV diagnosis than those who had not received a HIV diagnosis (33% vs. 9%, $P = 0.02$).

In conclusion, HIV-infected males accounted for 30% of Taiwanese male EHI cases who reported recent travel to *E. histolytica*-endemic areas and a substantial proportion (21%) of males with EHI subsequently received an HIV diagnosis. One-third of HIV, *E. histolytica*-coinfected male travelers and almost 10% of those monoinfected with *E. histolytica* had engaged in oral, anal, or oral–anal sex, suggesting the need to consider and counsel for EHI not only as a foodborne or waterborne disease but also a sexually transmitted infection for male travelers with HIV infection or sexual risks [4].

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

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Abbreviations: EHI, *Entamoeba histolytica* infection.

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Optimal Dose of Benzathine Penicillin G for the Treatment of Early Syphilis in HIV-Infected Patients in the Era of Combination Antiretroviral Therapy

To the Editor—We read with interest the article by Ganesan et al [1] that addresses a key question in syphilis management regarding the optimal dose of benzathine penicillin G (BPG) for the treatment of early syphilis in human immunodeficiency virus (HIV)-infected patients [2]. In this retrospective cohort study of 350 Department of Defense beneficiaries with 393 episodes of early syphilis between 1986 and 2013, the authors found that treatment with 1 dose and 2 or more doses of BPG resulted in a similar rate of a ≥4-fold decline of nontreponemal antibody titers in 141 and 252 cases of early syphilis, respectively, at 13 months of follow-up (92% vs 92%). The study spanned >20 years, during which time the sexually transmitted diseases treatment guidelines have evolved [3] and combination antiretroviral therapy has been introduced. One-fourth of the patients in this study contributed >1 episode of early syphilis, for which different treatment regimens or doses of BPG might be administered for the subsequent episodes. Comparisons of the baseline characteristics of the 2 treatment groups are not shown in the article, which precludes assessment of any confounding factors or bias involved. Furthermore, whether the patients who might have received oral antibiotics for reasons other than syphilis during the follow-up were excluded is not described. Furthermore, limited by the case number, the study was not powered to draw a conclusion that the serological response rates to the 2 different doses of BPG were similar in HIV-infected patients with early syphilis.

The serological response rate in the study by Ganesan et al [1] is significantly higher than that reported in our recently published multicenter prospective cohort study that included 573 HIV-infected patients with early syphilis, in which 198 of 295 patients (67.1%) received 1 dose and 208 of 278 (74.8%) received 3 weekly doses of BPG [4]. In our study, the 2 treatment groups were similar in the clinical characteristics that have been reported to be associated with treatment response, and the sample size was of sufficient power to demonstrate the noninferiority of 1 dose to 3 weekly doses of BPG. At 12 months of treatment, however, we failed to demonstrate that 1 dose was noninferior to 3 doses of BPG given the finding that the 1-sided 95% confidence interval of the difference (15.1%) exceeded the predefined noninferiority margin (10%) [4]. The discrepancy of treatment responses between the study by Ganesan et al and ours may be related to the study design, subjects included for analysis, and definition for treatment failure. In our study, 94% of the patients were men who have sex with men, a population that has been identified as a high-risk group for treatment failure from reinfection [5]. Indeed, we included in the definition of treatment failure those who had an increase of rapid plasma reagin (RPR) titer after ever achieving a ≥4-fold decline within 12 months of treatment with 1 or 3 doses of BPG because it was difficult to differentiate relapse from reinfection solely based on the changes of RPR titers in this observational study. However, a substantial proportion of patients presented with primary and/or secondary syphilis in either treatment group during follow-up, suggestive of reinfection [4]. Therefore, we might have underestimated the effectiveness of the 2 treatment regimens. When the data were analyzed using the proportion of the patients who could achieve a ≥4-fold decline of RPR titers following treatment, the response rate was 88.1% and 86.0% for the 1-dose and 3-dose groups, respectively.

Both the study by Ganesan et al [1] and our study [4] are observational in design and subject to bias [6]. Although the optimal dose of BPG for early syphilis among HIV-infected patients remains unclear until randomized controlled clinical trials that use improved diagnostic modalities to reflect disease activity of syphilis are available to inform clinical decisions, we already have observed that the proportion of HIV-infected patients given 3 doses of BPG in our study has decreased from 60.2% (133/221) in 2007–2009 to 39.3% (53/135) in 2011 and 25.0% (12/80) in 2012 after the sexually transmitted diseases treatment guidelines recommended a single dose of BPG for the treatment of early syphilis in HIV-infected patients in 2010 [3]; besides, no cases of neurological complications suggesting neurosyphilis have been detected after follow-up for ≥3 years [4]. We believe that, until randomized clinical trials are available to address this key question, more observational studies among different risk groups will be beneficial in providing more insight into this dilemma [7].

Note

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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RESEARCH ARTICLE

Risk of Active Tuberculosis in HIV-Infected Patients in Taiwan with Free Access to HIV Care and a Positive T-Spot.TB Test

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Abstract

Background

Interferon-gamma release assays (IGRAs) have been used to identify individuals at risk for developing active tuberculosis (TB). However, data regarding the risk of TB development in HIV-infected patients testing positive for IGRAs remain sparse in the era of combination antiretroviral therapy.

Methods

Between 2011 and 2013, 608 HIV-infected patients without active TB undergoing T-Spot.TB testing were enrolled in this prospective observational study at a university hospital designated for HIV care in Taiwan with a declining TB incidence from 72 per 100,000 population in 2005 to 53 per 100,000 population in 2012. All of the subjects were followed until September 30, 2014. The national TB registry was accessed to identify any TB cases among those lost to follow-up.

Results

T-Spot.TB tested negative in 534 patients (87.8%), positive in 64 patients (10.5%), and indeterminate in 10 patients (1.6%). In multivariate analysis, positive T-Spot.TB was significantly associated with older age (adjusted odds ratio [AOR], 1.172 per 10-year increase; 95% confidence interval [CI], 1.022-1.344, P=0.023), past history of TB (AOR, 13.412; 95% CI, 6.106-29.460, P<0.001), and higher CD4 counts at enrollment (AOR, per 50-cell/μl increase, 1.062; 95% CI, 1.017-1.109, P=0.007). Of the 64 patients testing positive for T-Spot.TB, none received isoniazid preventive therapy and all but 5 received combination antiretroviral therapy at the end of follow-up with the latest CD4 count and plasma HIV RNA load being 592.8 cells/μL and 1.85 $\log_{10}$ copies/mL, respectively. One patient (1.6%) developed active

Competing Interests: The authors have declared that no competing interests exist.

TB after 167 person-years of follow-up (PYFU), resulting in an incidence rate of 0.599 per 100 PFYU. None of the 534 patients testing negative for T-Spot.TB developed TB after 1380 PYFU, nor did the 24 patients with old TB and positive T-Spot.TB tests develop TB after 62.33 PYFU.

Conclusions

The risk of developing active TB in HIV-infected patients with positive T-Spot.TB receiving combination antiretroviral therapy is low in Taiwan where the national TB program has led to a sustained decrease in TB incidence.

Introduction

The lifetime risk for active tuberculosis (TB) is estimated to be 5 to 10% for a person with a positive tuberculin skin test (TST) [1], but the risk is much higher in HIV-infected patients, 10% per year [2]. Because interferon-gamma (IFN-γ) plays a pivotal role in regulating cell-mediated immune response against TB, interferon-gamma release assays (IGRAs) were developed to detect *Mycobacterium tuberculosis* infection [3–5]. The Food and Drug Administration (FDA)-approved IGRAs include QuantiFERON-TB (QFT), QuantiFERON-TB Gold test (QFT-G), QuantiFERON-TB Gold In-Tube test (QFT-GIT), and T-Spot.TB [6]. The Centers for Disease Control and Prevention (CDC) recommend IGRAs be used as TST as aids in diagnosing infection with *M. tuberculosis*, and IGRA is preferred particularly for persons that have low rates of returning to have TST read or those who have received BCG vaccination [6].

After IGRAs were available, numerous studies have been conducted to further characterize the use of IGRAs. One fixed-effects meta-analysis of 20 studies assessed the performance of IGRAs [7], which showed that the pooled sensitivity was 78% (95% confidence interval [CI], 73% to 82%) for QFT-G, 70% (95% CI, 63% to 78%) for QFT-GIT, and 90% (95% CI, 98% to 100%) for T-Spot.TB [7]. The meta-analysis concluded that T-Spot.TB appeared to be more sensitive than both QuantiFERON tests and TST [7].

Because IGRAs detect a cellular immune response to *M. tuberculosis*, IGRAs have reduced sensitivity in immunocompromised patients. In a systematic review of 37 studies including 5736 HIV-infected individuals, the pooled sensitivity estimates were higher for T-Spot.TB (72%; 95% CI, 62%-81%) than for QFT-GIT (61%; 95% CI, 41%-75%), but none of the IGRAs were consistently more sensitive than TST in head-to-head comparisons. Although T-Spot.TB appears to be less affected by immunosuppression than QFT-GIT and TST, the overall differences among these three tests are small or inconclusive [8]. Another systematic review of 38 studies including 6514 patients demonstrated that pooled indeterminate rates were higher in high TB burden settings than in settings of low to intermediate burden and in patients with CD4 lymphocyte counts $<$200 cells/μL [9].

Given the fact that there is no gold standard for latent TB infection, the only way to confirm the predictive value for progression to TB of TST or IGRAs is the development of active TB in tested persons during follow-up. Two meta-analyses showed different results; one concluded that neither IGRAs nor TST had high accuracy for the prediction of active TB [10], but the other demonstrated that commercial IGRAs had a higher positive as well as negative predictive value for progression to active TB compared with TST [11]. Such discrepancies might result from different inclusion criteria; one included only persons without IPT for analysis [11] while the other did not have such a criterion [10]. Analytic approach adopted also explains such

discrepancies; one did not take duration of follow-up into consideration [11], but the other used incidence rates to present the prognostic capability of the IGRAs [10]. Nevertheless, such data in HIV-infected population remain limited [12–16]. Thus, the present study aimed to assess the risk for progression to active TB in HIV-infected patients testing for T-Spot.TB through longitudinal follow-up.

Materials and Methods

Setting and Study population

Taiwan has an estimated TB incidence of 53.0 per 100,000 population in the general population in 2012, which has decreased from 72.0 per 100,000 in 2005 with the implementation of the national TB control program aiming to halve TB incidence by 2015 [17]. To make this achievement possible, the national TB control program launched a directly observed treatment short-course program (DOTS) for all identified TB patients, scaling-up of laboratory diagnosis, contact investigation, and treatment of latent TB infection. HIV-infected patients in Taiwan are provided free-of-charge access to HIV care, including combination antiretroviral therapy (cART) that was introduced on April 1, 1997 and monitoring of CD4 lymphocyte counts and plasma HIV RNA loads at designated hospitals around the island. In this study, we enrolled HIV-infected patients aged 20 years or greater who sought HIV care between March 2011 and September 2013 for detection of latent TB infection using T-SPOT.TB assay at the National Taiwan University Hospital. During the study period, isoniazid preventive therapy (IPT) was suggested for patients with positive results, but was initiated at the discretion of primary care physicians after discussion with the patients.

All enrolled patients had regular follow-up as outpatients every 3 to 6 months. Work-up, including chest radiography, acid-fast staining, mycobacterial cultures, and histopathology of the infected sites would be performed if the patients had symptoms or signs highly suspected of TB during the follow-up. The protocol was approved by the Research Ethics Committee of the National Taiwan University Hospital (registration number, 201103060RC) and all patients provided written informed consent prior to enrollment.

Data collection

A computerized data collection form was used to retrieve the demographic and clinical data of HIV-infected patients from the medical records, including age, gender, risk behaviors for HIV transmission, CD4 count and plasma HIV RNA load, and TB status, active or old. Old TB was considered if the information of the national TB reporting website (https://tb.cdc.gov.tw/slow/CA/LoginByCard.asp) showed that the patient had a history of active TB reported 3 months or greater before the study enrollment, and there were no clinical symptoms and signs of active TB at enrollment. Active TB was considered if the information of the national TB reporting website showed that the patient had a history of active TB reported within 3 months of the study enrollment, and there were clinical symptoms and signs of active TB or culture- or histopathology-confirmed TB within 3 months of the study enrollment. Cross-matching with the national TB reporting website was performed at the end of the follow-up to obtain the data from any patients who were lost to follow-up at this hospital during the study period.

Laboratory investigations

T-SPOT.TB assay (Oxford Immunotech Ltd., Oxford, UK) was performed to detect latent TB infection in HIV-infected patients, and the results were interpreted according to the CDC's criteria [6]. HIV infection was diagnosed by detection of anti-HIV antibodies using enzyme-linked

immunosorbent assay or particle agglutination (SFD HIV 1/2 PA, Bio-Rad FUJIREBIO, Japan) and confirmed by Western Blot test (MP Diagnostics HIV BLOT 2.2. MP Biomedicals Asia Pacific Pte Ltd, Singapore). Plasma HIV RNA load and CD4 lymphocyte count were quantified by the Cobas Amplicor HIV-1 Monitor Test, version 1.5, (Roche Diagnostics Corporation, Indianapolis, USA) and FACSFlow (BD FACS Calibur, Becton Dickinson, CA), respectively.

Statistical analysis

All statistical analyses were performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL). Categorical variables were compared using χ^2 or Fisher's exact test whereas non-categorical variables were compared using Student's t-test. A multivariable logistic model was used to estimate the effects of multiple variables on the T-Spot.TB positivity. A backward stepwise selection was used. All variables significant at $P<0.20$ in the univariate analysis were entered in the model and then removed in a stepwise design if $P>0.20$. Interactions among the main effects were examined, and the final model was checked with the Hosmer-Lemeshow goodness of fit test. The incidence rate of developing active TB during follow-up period was calculated as the number of episodes of active TB per 100 person-years of follow-up (PYFU). All tests were two-tailed and a P value <0.05 was considered significant. The TB status of enrolled HIV-infected patients was followed until the end of the study on September 30, 2014 or death before September 30, 2014, whichever occurred first.

Results

Between March 2011 and September 2013, a total of 617 HIV-positive patients were enrolled and followed until their death or September 30, 2014. After exclusion of 9 patients with active TB at enrollment, 543 patients (87.8%) had negative T-Spot.TB results, 64 (10.5%) positive results, and 10 (1.6%) indeterminate results (Fig 1). Of the 608 included study subjects, 81.0% were male homosexuals, 15.7% heterosexuals, 1.5% injecting drug users, and 1.8% others. The mean follow-up duration at the end of the study when TB status was evaluated was 2.57 years, and 15 patients (2.5%) died during the follow-up.

Compared with patients with negative T-Spot.TB results, those with positive T-Spot.TB results had a significantly higher mean (± standard deviation [SD]) CD4 count (578.4 ± 306.5 vs 441.6 ± 295.9 cells/μL) and lower mean (± SD) plasma HIV RNA load

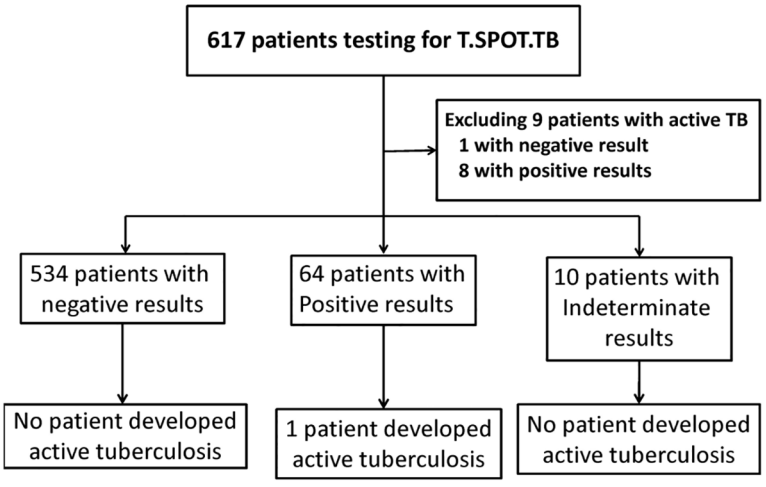


Fig 1. Enrollment and disposition of the study subjects.

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(2.35 ± 1.23 vs 2.89 ± 1.50 $\log_{10}$ copies/mL) and were significantly older (mean age [± SD], 45 ± 13.1 vs 36.9 ± 10.8 years), less likely to be male (90.6% vs 97.6%), and more likely to have a history of old TB (37.5% vs 3.7%) and to receive cART at the time of T-Spot.TB testing (84.4% vs 61.0%) (all *P* values <0.05) (Table 1). After a mean (± SD) follow-up duration of 943.3 ± 177.4 and 952.5 ± 98.8 days (*P* = 0.683) for patients with negative and those with positive T-Spot.TB results, respectively, those with positive T-Spot.TB maintained a higher CD4 count (592.8 ± 294.9 vs 451.9 ± 274.3 cells/μL, *P*<0.001) and lower plasma HIV RNA viral load (1.85 ± 0.97 vs 2.64 ± 1.50 $\log_{10}$ copies/mL, *P*<0.001) than those with negative T-Spot.TB despite the fact that similar proportions of the two groups were on cART (92.2% vs 90.1%, *P* = 0.589).

In multivariate analysis, we included variables with significant difference between the two groups in univariate analysis (Table 1), such as age, male gender, a history of old TB, CD4 count, plasma HIV RNA load at enrollment and cART use, and decided the final model by using the Hosmer-Lemeshow goodness-of-fit test. We found that the independent factors

Table 1. Demographic and clinical characteristics of HIV-infected patients with negative, positive, and indeterminate T-Spot.TB results.

Results of T-Spot.TB	Negative	Positive	Indeterminate	P[a] value	P[b] value
Patient No., n (%)	534 (87.8)	64 (10.5)	10 (1.6)		
Age, mean (SD)	36.9 (10.8)	45 (13.1)	41.1 (14.4)	<0.001	<0.001
Male gender, n (%)	521 (97.6)	58 (90.6)	10 (100.0)	0.023	0.009
Risk for HIV transmission, %					
Homosexuals	83.2	70.6	71.4	0.056	0.077
Heterosexuals	13.6	27.5	14.3		
Injecting drug users	1.4	2.0	0		
Others	1.8	0	14.3		
At T-Spot.TB testing, n (%)					
Previous TB, n (%)	20 (3.7)	24 (37.5)	0 (0)	<0.001	<0.001
CD4 lymphocyte count, mean (SD), cells/μl	441.6 (295.9)	578.4 (306.5)	473.5 (316.0)	<0.001	0.001
CD4 <200, n (%)	93 (18.5)	10 (14.5)	1 (11.1)	0.200	0.422
PVL, mean (SD), $\log_{10}$ copies/mL	2.89 (1.50)	2.35 (1.23)	2.79 (1.84)	0.005	0.003
PVL >5 $\log_{10}$ copies/mL, n (%)	59 (11.8)	1 (1.7)	1 (11.1)	0.029	0.019
PVL <50 copies/mL, n (%)	223 (44.5)	37 (63.8)	6 (66.7)	0.009	0.005
Newly diagnosed HIV infection, n (%)	55 (10.4)	4 (6.3)	1 (10.0)	0.36	0.315
On cART, n (%)	326 (61.0)	54 (84.4)	6 (60.0)	0.001	<0.001
End of follow-up					
CD4 lymphocyte count, mean (SD), cells/μl	451.9 (274.3)	592.8 (294.9)	429.9 (319.3)	0.003	<0.001
CD4 <200, n (%)	87 (16.7)	4 (6.3)	3 (30.0)	0.031	0.030
PVL, mean (SD), $\log_{10}$ copies/mL	2.64 (1.50)	1.85 (0.97)	1.94 (1.42)	<0.001	<0.001
PVL >5 $\log_{10}$ copies/mL, n (%)	48 (9.2)	1 (1.6)	1 (10.0)	0.069	0.037
PVL <50 copies/mL, n (%)	251 (48.2)	46 (71.9)	7 (70.0)	<0.001	<0.001
On cART, n (%)	481 (90.1)	59 (92.2)	10 (100)	0.739	0.589
Duration of follow-up, mean (SD), person-days	943.3 (177.4)	952.5 (98.8)	868.8 (392.7)	0.586	0.683
Loss to follow-up, n (%)	66 (12.4)	6 (9.4)	0 (0)	0.647	0.488
Death, n (%)	13 (2.4)	0 (0)	2 (20.0)	0.048	0.464

Note:

[a] Comparison among patients with negative, positive, and indeterminate T-Spot.TB results

[b] Comparison between patients with negative and positive T-Spot.TB results

Abbreviations: cART, combination antiretroviral therapy; PVL, plasma HIV RNA load; SD, standard deviation; TB, tuberculosis

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associated with positive T-Spot.TB results were older age (adjusted odds ratio [AOR], per 10-year increase, 1.172; 95% confidence interval [CI], 1.022–1.344, $P = 0.023$), past history of TB (AOR, 13.412; 95% CI, 6.106–29.460, $P<0.001$), and higher CD4 counts at enrollment (AOR, per 50-cell/μl increase, 1.062; 95% CI, 1.017–1.109, $P = 0.007$) (Table 2).

Of the 64 patients with positive T-Spot.TB, 24 patients (37.5%) had a history of old TB with a mean interval of 2556 days (range, 209 to 5187 days) between T-Spot.TB testing and reporting date of TB. None of the 64 patients received IPT; all but 5 patients continued to receive cART at the end of follow-up. The latest mean CD4 count and plasma HIV RNA viral load at the end of follow-up of the 64 patients were 592.8 cells/μL and 1.85 $\log_{10}$ copies/mL, respectively. A diagnosis of active TB was made in 1 patient (1.6%) after a follow-up of 337 days who was receiving cART with a CD4 count of 78 cells/μL and plasma HIV viral load of 98 copies/mL. The incidence rate of TB in these 64 patients with positive result was 0.599 per 100 PYFU after a total follow-up of 167 person-years, and 0.064 per 100 PYFU in 608 enrolled patients without active TB at baseline after a total follow-up duration of 1571 person-years. None of the 24 patients with previous TB who tested positive for T-Spot.TB developed active or recurrent TB after a total follow-up duration of 62.33 person-years. Thus, the positive predictive value for developing active TB in those testing positive for T-Spot.TB was only 1.6% (1/64).

Of 534 patients with negative T-Spot.TB results, 20 patients (3.7%) had a history of old TB with a mean interval of 3117 days (range, 141 to 6361 days) between T-Spot.TB testing and reporting date of TB. None of the 534 patients developed active TB after a total follow-up of 1380 person-years, and 481 (90.1%) of them received cART at the end of follow-up with the latest mean CD4 and plasma HIV RNA load of 451.9 cells/μL and 2.64 $\log_{10}$ copies/mL, respectively. Thus, the negative predictive value for developing active TB in those testing negative for T-Spot.TB was 100%.

Of the 10 patients with indeterminate T-Spot.TB, their median CD4 counts were 458.8 cells/μL (range, 0 to 934 cells/μL) and median plasma HIV RNA load 1.60 $\log_{10}$ copies/mL (range, 2 to 6 $\log_{10}$ copies/mL) at the time of T-Spot.TB testing; none had a history of TB. After a total follow-up of 23.8 person-years when all of the patients were on cART, none developed active TB with the latest median CD4 count of 438.6 cells/μL, and plasma HIV RNA load of 1.3 $\log_{10}$ copies/mL.

Discussion

In this study conducted in a country of moderate TB endemicity, we found that the prevalence of latent TB infection diagnosed by positive T-Spot.TB was 10.5% (64/608) in HIV-infected patients. One of the 64 patients with positive T-Spot.TB and without IPT developed active TB during follow-up, yielding a positive predictive value of TB progression of 1.6%. The incidence rate of active TB was 0.598 per 100 PYFU in 64 patients with positive T-Spot.TB and 0.064 per

Table 2. Multivariate analysis for factors associated with positive T-Spot.TB results.

Variables	Reference	Odds ratio	95% confidence interval	*P* value
Age (per 10-year increase)	Continuous variable	1.172	1.022–1.344	0.023
Male	Female	0.399	0.104–1.533	0.181
Old TB	No old TB	13.412	6.106–29.460	<0.001
CD4 counts at enrollment (per 50-cell/μl increase)	Continuous variable	1.062	1.017–1.109	0.007
Plasma HIV RNA load at enrollment (per 1-$\log_{10}$ increase)	Continuous variable	0.804	0.629–1.028	0.082

Abbreviations: PVL, plasma HIV RNA load; TB, tuberculosis

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100 PYFU in 608 patients without active TB at baseline. None of the patients with negative T-Spot.TB developed active TB, which resulted in a negative predictive value of 100%.

The incidence rate and crude incidence of active TB in HIV-infected patients with positive IGRA results range from 0 to 4.621 cases per 100 person-years and from 0 to 20%, respectively (summarized in Table 3) [12–16], depending on the TB epidemiology of the country where the study was conducted, number of study subjects enrolled (range, 50 to 909 subjects), follow-up duration (range, 1.28 to 2.97 years), methods employed to detect and diagnose TB, receipt of IPT, CD4 counts (range, median, 305 to 447 cells/μL) and plasma HIV RNA loads (range, 1.85 to 4.8 $\log_{10}$ copies/mL), and the proportion of study subjects on cART (range, 0 to 100%).

The incidence rate or crude rate in our patients with positive IGRA results appears to be lower than those reported in studies conducted in countries of different levels of TB endemicity (Table 3) [12–16, 18]. A recent study conducted in Brazil (TB incidence of 46 per 100,000 population [19]) reported a TB incidence of 6.52 cases per 100 PYFU in HIV-infected patients with positive TST and without IPT [18]. Compared with the study in Brazil [18], our study cohort had a higher cART coverage (84.4% vs. 53%), higher CD4 counts at enrollment (578.4 vs. 447 cells/uL) and a shorter follow-up duration (2.6 vs. 4.7 years), which may contribute to the observed difference in the incidence rate of active TB.

In the study conducted in Taiwan in which 40% of the subjects were incarcerated injecting drug users [13], Yang et al found an overall rate of 4.7% and an incidence rate of 1.72 per 100 PYFU of active TB in 909 subjects enrolled despite the fact that 87 of 227 patients (38.3%) with positive TST received IPT. Other than the differences in the composition and HIV disease status of the study subjects, the background TB incidence may also play a role in the development of active TB. Our study and that by Yang et al. were conducted over two different study periods

Table 3. Comparisons of progression of tuberculosis in HIV-infected patients with interferon-gamma release assay testing.

Reference	Country	IGRA	[1]Patient No.	Positive IGRA, n (%)	Indeterminate IGRA, n (%)	Follow-up duration	No. of [2]TB cases; incidence (%); [3]incidence rate (cases/100 PY)	IPT	Median CD4, cells/uL	Median PVL, $\log_{10}$ copies/mL	cART, %
Presentstudy	Taiwan	T-Spot.TB	609	64 (10.5)	10 (1.6)	2.57 years	1; 1.6; 0.598	No	437	1.85	63.5
[13]	Taiwan	T-Spot.TB	909	134 (14.7)	0 (0)	2.97 years	[4]4; [4]4.7; [4]1.72	[5]Yes/No	447	3.25	40.6
[16]	China	T-Spot.TB	50	21 (42)	NA	36 months	0; 0; 0	No	305	NA	100
[15]	Korea	T-Spot.TB	120	30 (25)	7 (5.8)	32 months	6; 20; 4.621	No	306	NA	36.7
[14]	Kenya	T-Spot.TB	333	120 (36.4)	52 (15.6)	1.28 years	6; 5; 4.2	No	440	4.8	0
[12]	Austria	GFT-GIT	822	37 (4.5)	47 (5.7)	19 months	3; 8.1; NA	No	393	NA	59.6

Note:

[1]Patient No.: patient number after exclusion of patients with active TB at enrollment;

[2]TB cases, cases of patients with positive IGRA results who developed TB during follow-up;

[3] The incidence rate of patients with positive IGRA results who developed TB during follow-up;

[4]Individuals with isoniazid prophylaxis therapy were excluded;

[5]Yes/No: 4 of 32 patients with positive IGRA results receiving no IPT developed TB and 1 of 49 patients with positive IGRA results receiving IPT developed TB;

Abbreviations: cART: combination antiretroviral therapy; IGRA, interferon-gamma release assay; IPT: isoniazid preventive therapy; NA: not available; PY, person-years; TB, tuberculosis

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(2011–2013 vs. 2008–2010), during which time the national TB incidence has decreased from 73 in 2005 to 53 per 100, 000 population in 2012 with the implementation of the intensified national TB programs launched in 2006 to halve the TB incidence by 2015 [17].

While the incidence rate of our HIV-infected patients testing positive for T-Spot.TB is lower than that reported in many other studies (Table 3), the rate (0.598 per 100 PYFU) remains significantly higher as compared to that in the general population despite free access to cART and sustained improvement of immunity with cART in our patients. To further decrease the risk of progression to TB among HIV-infected patients in Taiwan, earlier initiation of cART and IPT are the two strategies that have been confirmed to be beneficial in several clinical trials [20–23]. However, a substantial proportion of our HIV-infected patients presented late for HIV care [24, 25]. More efforts are needed to facilitate early diagnosis of HIV infection and to overcome the obstacles to linkage to and retention in HIV care, prescription of cART and achievement of sustained suppression of HIV replication [26].

Identification of latent TB infection and implementation of IPT has been recommended to achieve sustained decreases of TB incidence by an expert panel of review of the national TB program in Taiwan [17]. Such a recommendation could also be applied to HIV-infected patients as a randomized double-blind placebo-controlled trial that has demonstrated that all patients receiving antiretroviral therapy in moderate or high incidence areas should receive IPT regardless of the status of TST or IGRAs [23]. While concerns about the durability of IPT remains [27, 28], a recent Brazilian study demonstrates that the benefit of IPT may last 7 years in the cART era in a country with moderate TB endemicity [18].

Several limitations of our study deserve to be acknowledged. First, 80.9% of the study subjects were male homosexuals, and only 1.8% were injecting drug users. Our results may not be generalizable to injecting drug users with HIV infection who have been identified as a high-risk group for active TB [29]. Second, not all patients received regular work-up for TB during follow-up, though when the clinical index of suspicion was high, patients underwent extensive TB work-up. Third, 12.3% of the patients with positive IGRA results were lost to follow-up. Nevertheless, as we had access to the national TB registry and TB is a mandatory notifiable disease, data regarding the development of TB in those lost to follow-up were available. Fourth, we were not able to assess the risk factors for TB progression in those with positive IGRA results because only one patient developed TB during follow-up. Fifth, the follow-up duration in our study remains short. With ageing and increasing rates of morbidities in patients with prolonged exposure to cART in Taiwan [30], the risk for developing active TB warrants further investigation. Lastly, the study was conducted in a country of moderate TB endemicity and the results may not be generalizable to countries of higher TB endemicity where cART program may not be widely implemented.

In conclusion, the risk of developing active TB in HIV-infected patients positive for T-Spot.TB receiving cART is low in a country of moderate endemicity of TB as compared with that in other countries of similar endemicity.

Supporting Information

S1 Data. Raw data of HIV-infected patients testing for T-SPOT.TB.
(XLS)

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Author Contributions

Conceived and designed the experiments: H-YS P-RH S-YC C-CH S-CC. Performed the experiments: P-RH W-CL Y-CS. Analyzed the data: H-YS S-YC C-CH. Contributed reagents/materials/analysis tools: P-RH S-YC S-CC. Wrote the paper: H-YS P-RH C-CH S-CC.

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Detection of human parvovirus 4 viremia in the follow-up blood samples from seropositive individuals suggests the existence of persistent viral replication or reactivation of latent viral infection

Mao-Yuan Chen*, Chien-Ching Hung and Kuang-Lun Lee

Abstract

Background: The transmission routes for human parvovirus 4 (PARV4) infections in areas with high seroprevalence are not known. In the work described here, persistent PARV4 viral replication was investigated by conducting a longitudinal study.

Methods: Ten healthcare workers each provided a blood sample at the beginning of the study (first sample) and 12 months later (second sample). The paired samples were tested for PARV4-positivity by immunoblotting analysis and nested polymerase chain reactions.

Results: IgG antibodies against PARV4 were detected in six participants, three of whom also had IgM antibodies against PARV4. The immunoblotting results did not vary over time. PARV4 DNA was detected in the first blood sample from one participant who had IgG antibodies against PARV4 and in the second blood samples from 2 participants who had IgG and IgM antibodies against PARV4.

Conclusions: Detection of PARV4 DNA in the second blood samples from two seropositive participants suggests the existence of persistent PARV4 replication or reactivation of inactive virus in the tissues. The finding of persistent or intermittent PARV4 replication in individuals with past infections provides an important clue toward unraveling the non-parenteral transmission routes of PARV4 infection in areas where the virus is endemic.

Keywords: Parvovirus 4, Persistent PARV4 infection, Latent PARV4 infection, Anti-PARV4 IgM

Background

In the subfamily *Parvovirinae* of the family *Parvoviridae*, members of four genera are currently known to infect humans, namely parvovirus B19 (B19V, genus *Erythroparvovirus*), human bocavirus (genus *Bocaparvovirus*), adeno-associated viruses (genus *Dependoparvovirus*) and human parvovirus 4 (PARV4, genus *Tetraparvovirus*); of these, the former two viruses can cause human diseases [1]. PARV4 was discovered in an intravenous drug user with an acute viral infection syndrome [2]. However to date, the clinical manifestations of PARV4 infection remain largely unknown. The seroprevalence of PARV4 varies geographically, but antibodies against PARV4 have been detected in 24.8 % of the general population in Cameroon in contrast to none of the general population in the United Kingdom [3]. Early serological studies supported a predominantly parenteral route for PARV4 infection in northern Europe because the seropositivity rates were 0 % and 0.9 % in the study groups where a risk of parenteral infection was not found [4, 5]. By contrast, recent studies found that 9.4 % of a low-risk group in Lithuania and 4.76 % of United Kingdom blood donors tested positive for PARV4 antibodies [6, 7]. How the study subjects in these two reports [6, 7] acquired PARV4 infection is not known.

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The strong immune response induced by acute B19V infection can eliminate B19V viremia. Persistent B19V viremia does exist [8] but is infrequently found and characterized by a low viral load. However, B19V DNA frequently remains detectable in tissues long after viral exposure, perhaps even after a lifetime [9]. Similarly, PARV4 viremia was estimated to last only between 32 and 104 days in study subjects who displayed seroconversion [10] and PARV4 DNA could also be detected in 15 to 41 % of the liver, myocardium, lung and kidney tissues from non HIV-infected individuals in the absence of viremia which suggests persistence of viral DNA in tissues [11, 12]. Not every study found PARV4 DNA in tissue samples from non HIV-infected study subjects [13], although B19V and PARV4 DNA were detected in 54 % and 71 % of autopsy tissue samples, respectively, from HIV-infected study subjects in the same study [13]. However, both B19V and PARV4 DNA were absent in the plasma of 36 previously untreated HIV-infected patients despite the high probability of viral DNA persistence in tissue samples [13]. These findings raise the question of whether these viruses in tissues have the ability to actively replicate. In addition, the detection rate of B19V DNA in plasma was low (0.9 %) in blood donors from the United Kingdom despite 60.5 % of them being positive for anti-B19V IgG [14]. This suggests that most of the blood donors with past B19V infections do not have active B19V replication in their tissues or have replication levels below detection limit. In the case of PARV4, it was reported that the PARV4 DNA detection rate in blood donors from Los Angeles (USA) was low (2 %); however, the PARV4 seropositive rate is not known [15]. Although no direct evidence to date has supported persistent PARV4 replication in the host, the observation of sustained T cell responses against PARV4 without viremia suggests PARV4 may persist at levels below detection limit [10, 16].

In our previous report, the seven sequential blood samples collected from one HIV-infected male over a period of 21 months all tested positive for anti-PARV4 IgM but PARV4 DNA was found only in the second blood sample [17]. The patient might have had persistent PARV4 viremia, but the viral load was below the detection limit of the test method as has been reported previously in cases of persistent B19V viremia [8]. However, the possibility of re-infection could not be excluded because this was an immune-compromised host. To investigate the existence of PARV4 replication in healthy individuals with past PARV4 infections, we conducted the present study.

Methods

Study group

In this longitudinal study, ten healthcare workers (nine females) in our AIDS Center (National Taiwan University Hospital, Taiwan) donated blood on two occasions, with each donation 12 months apart. Their ages at the time of the first donation are listed in the Table. For each participant, 5 ml of blood was collected in an EDTA tube and the plasma was stored at −30 °C. The PARV4 serological status of these participants was unknown to us when they enrolled in the study. The investigation was carried out in accordance with the principles of the Declaration of Helsinki, and all of the participants provided informed consent. The collections of blood samples were approved by the ethics committee of the National Taiwan University Hospital.

Immunoblotting and nested polymerase chain reaction (PCR)

Antibodies against PARV4 were detected using an immunoblotting method developed by our group [17]. Two of the recombinant fusion proteins used, SUMOVP2 (amino acids 272–630 of the translated PARV4 open reading frame, ORF2) and SUMOVP3 (amino acids 604–914 of the translated ORF2), and the control protein have been described in our previous study [17]. Here, an additional fusion protein SUMOVP1 (amino acids 1–275 of the translated ORF2) was generated by the methods used in our previous report [17]. The immunoblotting assays were analyzed by two independent researchers (blinded), and samples with reactivity to fusion protein VP1, VP2, or VP3 were considered seropositive.

PARV4 DNA was detected using nested PCR. Viral DNA was extracted from 200 μL of plasma, using the High Pure Viral Nucleic Acid Kit (Roche, Mannheim Germany). The elution volume was 50 μL, and 5 μL was used in each reaction. The PARV4 primers (designed to detect PARV4 genotype 2) have the following sequences: forward: 5′-ATTTCTGTAGCTCCACCAGGAGC-3′ (nucleotide, nt.316 to 338, the numbers represent the nucleotide position in ORF2 and ATG is nt. 1), reverse: 5′-CCATAAACCTC TAATAGCATTGCC-3′ (nt.903 to 880), inner forward: 5′-CAAAAGCACCAAATGCTGAAAG-3′ (nt.380 to 401), and inner reverse: 5′- GGGAATCAGCTCCTTCATCG CG-3′ (nt.610 to 589). The thermo-cycling conditions for nested PARV4 PCR in the first and second rounds were 94 °C for 2 min, followed by 36 cycles of 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 30 s with a final extension of 7 min at 72 °C. PCR products were validated by sequencing and each DNA sample was tested using nested PCR six times, with the PARV4 ORF1 DNA template being included in each of 12 PCR experiments in this study as a negative control. The PCR experiments were repeated because it has been shown that PCR replicates are required to identify persistent B19V viremia when the B19V viral load is lower than the detection limit [8]. When six PCR replicates were performed on DNA samples with concentrations of 1, 0.5 or 0.25 PARV4

plasmid copies/μL, the number of positive results amounted to 6, 2 or 0, respectively.

Results and discussion

The serological and PCR results from the longitudinal study are listed in Table 1. Notably, the immunoblotting results for the 10 study participants in this study did not change over time. Anti-PARV4 IgM persisted without weakening in all three of the IgM-positive participants and IgG reactivity also remained constant (participant 9 and 10 are shown in Figs. 1 and 2, respectively). The IgG reactivity of participant 9 was so weak that it could hardly be detected after scanning (Fig. 2). One blood sample collected from participant 9 at 32 months before the start of the study also had the same immunoblotting results (strip not shown). We cannot explain the persistence of IgM reactivity with weak IgG reactivity noted in participants 9 and 10 (both were PARV4 DNA-positive)

Table 1 Longitudinal serological and PCR data

Participant	Sex	Age	Sample	Anti-PARV4 immunoglobulin	No. of positive results in six PCR replicates
1	M	38	1	Negative	0/6
			2	Negative	0/6
2	F	45	1	Negative	0/6
			2	Negative	0/6
3	F	24	1	Negative	0/6
			2	Negative	0/6
4	F	36	1	Negative	0/6
			2	Negative	0/6
5	F	26	1	IgG positive	0/6
			2	IgG positive	0/6
6	F	32	1	IgG positive	0/6
			2	IgG positive	0/6
7	F	28	1	IgG weakly positive	1/6
			2	IgG weakly positive	0/6
8	F	25	1	IgG & IgM positive	0/6
			2	IgG & IgM positive	0/6
9	F	30	1	IgG weakly positive & IgM positive	0/6
			2	IgG weakly positive & IgM positive	2/6
10	F	24	1	IgG & IgM positive	0/6
			2	IgG & IgM positive	1/6

M male, *F* female, *No.* number

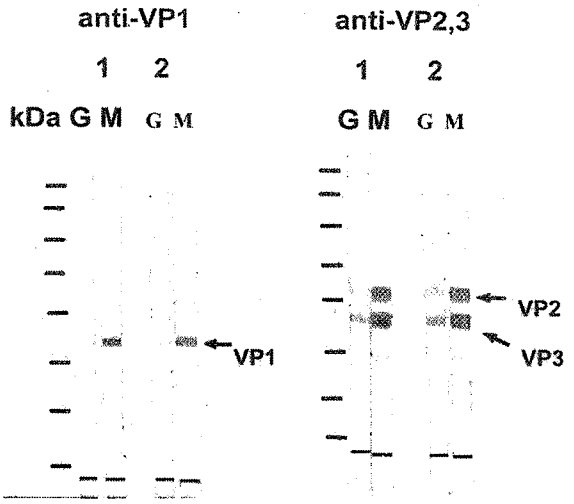


Fig. 1 PARV4 immunoblots from study participant No. 10. The two blood samples collected from this subject 12 months apart are represented by 1 and 2. PARV4 DNA was detected in the second but not the first blood sample. Note the strong IgM and weak IgG reactivity over the study period. VP1 represents the fusion protein SUMOVP1 (a.a. 1–275 of the translated PARV4 open reading frame, ORF2); VP2 represents SUMOVP2 (a.a. 272–630 of translated ORF2) and VP3 represents SUMOVP3 (a.a. 604–914 of translated ORF2). The molecular weight markers (kDa) are 170, 130,100, 70, 55, 40, 35 and 25 from top to bottom of each blot. G: IgG; M: IgM; a.a.: amino acid

but this pattern is not uncommon in our experience and these subjects were likely to be diagnosed as having current PARV4 infection without knowledge of their preceding immunoblotting results. Interestingly, the PARV4 DNA-positive participant (number 7) who was negative for anti-PARV4 IgM also had weak IgG reactivity (Fig. 2). PARV4 DNA was detected only in the first sample (one participant) or in the second sample (two participants). The number of positive PCR replicates was one (two samples) or 2 (one sample). The three nucleotide sequences of the PCR products are identical to the corresponding region of the GenBank sequence GU120197.1.

Both B19V and PARV4 viruses have two capsid proteins, VP1 and VP2 [18], and antibodies against linear B19V VP2 epitopes have been shown to disappear 6 months after acute B19V infection [19]. In the present study, the three PARV4-SUMO fusion proteins SUMOVP1, SUMOVP2 and SUMOVP3 represent the PARV4 VP1-unique sequence, the PARV4 VP1-VP2 overlapping region, and the carboxyl terminal region of PARV4 VP2, respectively. In contrast to B19V, the antibodies against the linear epitopes of PARV4 VP2 did not disappear over time (Figs. 1 and 2).

To date, studies on the anti-PARV4 IgM response have yielded inconsistent results. A monthly follow-up study of intravenous drug users found that five drug users had seroconversion to PARV4, but four of them were positive for anti-PARV4 IgM only in the blood sample collected at

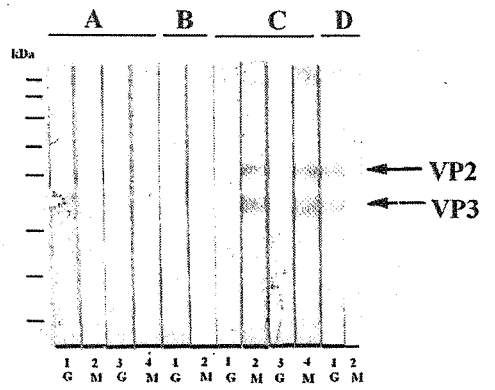


Fig. 2 Immunoblots of participants No. 7 (**a**), 1 (**b**), 9 (**c**) and 5 (**d**). Immunoblots of the first blood samples are 1 (IgG) and 2 (IgM), immunoblots of the second blood samples are 3 (IgG) and 4 (IgM). For participants 1 and 5, the immunoblots of the second blood samples are not shown. Participant 1 was negative for anti-PARV4 IgG and IgM, participants 7 and 5 were positive for anti-PARV4 IgG and participant 9 was positive for anti-PARV4 IgG and IgM. Note that the anti-PARV4 IgG reactivities of participant 7 and 9 are weak and can hardly be detected after scanning. Anti-PARV4 VP1 is not shown. VP2, VP3 and molecular weight markers are the same as in Fig. 1

seroconversion; the anti-PARV4 IgM became undetectable in the blood sample collected after a month [10]. For the one exception, two sequential blood samples tested positive for anti-PARV4 IgM, and the duration of the IgM response was 32–93 days. All the IgM-positive blood samples collected at seroconversion had detectable PARV4 DNA, but viremia disappeared in the second IgM-positive blood sample collected after one month and the duration of viremia was estimated to have lasted between 32 and 98 days. This result suggested that anti-PARV4 IgM and PARV4 viremia were both short-lived and the existence of PARV4 DNA was expected in most of the IgM-positive blood samples. In contrast, a serological study conducted in Finland found that four (5.1 %) HIV-infected intravenous drug users tested positive for anti-PARV4 IgM without having PARV4 viremia [5]. Furthermore, the presence of high-avidity anti-PARV4 IgG indicated that the four drug users had previous immunity rather than primary infection. Additionally, the anti-PARV4 IgM-positive rate was 7 % in a Lithuanian study on patients with acute respiratory disease in contrast to 0.9 % in a Finnish study on university students [5, 6]. These IgM-positive Lithuanian patients were grouped into current (IgM+, IgG−) ongoing, or recent PARV4 infection groups (IgM+, IgG+); however, PARV4 DNA was not detectable in any of the IgM-positive blood samples. In our experience, IgM antibodies against PARV4 can remain detectable for 12 months or longer, and the high IgM-positive rates are explainable by a prolonged IgM response, but PARV4 DNA might not have been detected in IgM-positive blood samples without conducting PCR replicates.

In the present study, the possibility of low-level viral infections in some subjects is suggested by the infrequent positive results of the PCR replicates, but false positive results seem unlikely because not a single negative control was positive (a total of 12 negative control reactions were performed in this study). Furthermore, the PARV4 DNA-positive blood samples in our PARV4 studies to date have always shown PARV4 antibody positivity with only one exception; this uneven distribution conflicts with the false positive results. A low viral load in the presence of anti-PARV4 IgG without IgM was noted in one participant (number 7 in the table), this was considered to result from persistent viral replication in a B19V study of blood donors [14]. Our study does not demonstrate persistent viral replication directly because PARV4 viremia was not found at more than one time point. Nevertheless, the detection of PARV4 DNA in the second of the two blood samples from two participants who were positive for anti-PARV4 IgM and IgG strongly suggests the existence of persistent PARV4 replication for the following reasons. (1) Primary PARV4 infection can be excluded because of the pre-existing anti-PARV4 IgM and IgG, which suggests the possibility of persistent PARV4 replication, reactivation of latent PARV4 infection or re-infection. (2) Re-infection can boost the pre-existing IgG antibody response, but this was not observed in the two study participants. (3) The low (1/35) seropositive rate in siblings of PARV4-infected hemophiliac patients [4] and the low PARV4 seroprevalence in United Kingdom blood donors [7] suggest that PARV4 is not easily transmitted by person-to-person contact through non-parenteral routes hence the chance of re-infection is low. (4) It is possible that persistent low-level viral replication below the detection limit existed in the first blood samples, but reappearance of viremia due to reactivation of inactive virus in the tissues is also possible.

Conclusions

The three PARV4 DNA-positive participants in this study were females of child-bearing age and persistent PARV4 replication is a possibility for them. If they become pregnant, PARV4 virus may be transmitted to their newborns, as was shown in our previous study [20]. Trans-placental PARV4 infection may be one of the major transmission routes in Taiwan because PARV4 seroprevalence in healthcare workers is high (43.6 %) [17], and 26 % of the seropositive individuals in our study of 31 healthcare workers were shown to have detectable PARV4 DNA by the method described herein (Chen MY, unpublished data). The hypothesis is also in agreement with the high PARV4 DNA-positive rates observed in Ghanaian infants

and children [21]. To address this issue, prospective studies on PARV4 infection in pregnant women and their newborns in PARV4 endemic areas are required.

Competing interests
The authors declare no competing interest regarding the present study.

Authors' contributions
CM performed laboratory tests and prepared the manuscript, HC and LG participated in the sample collection and data analysis. All authors have read and approved the final manuscript.

Authors' information
CM and LK are medical doctors at the Department of Internal Medicine, National Taiwan University Hospital (NTUH) in Taipei, Taiwan. HC works at the AIDS Center in NTUH and is an infectious disease specialist at NTUH.

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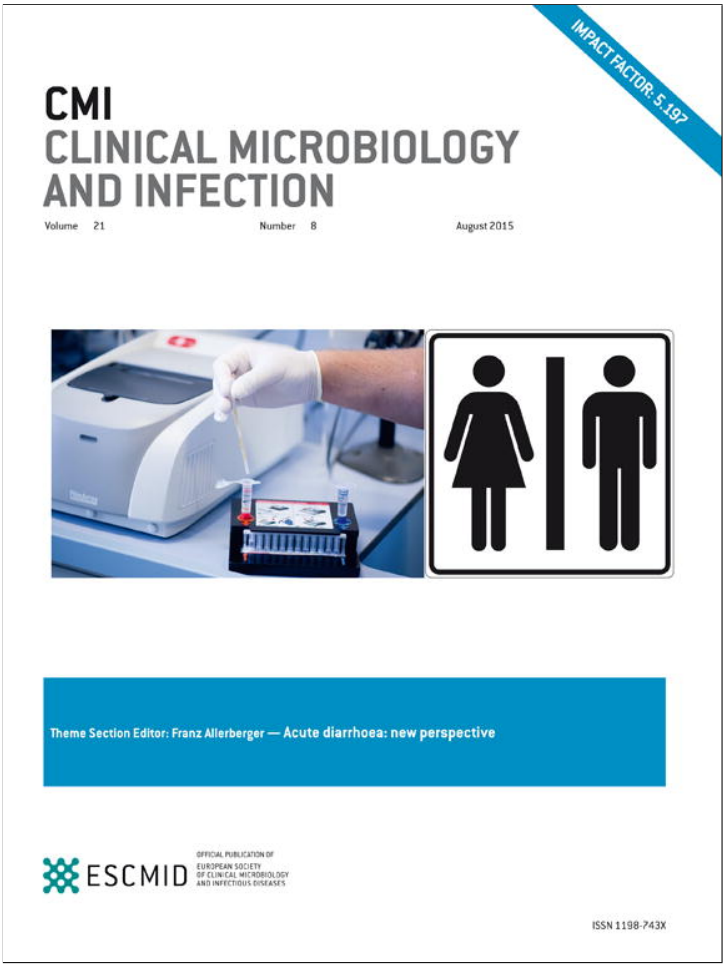
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ORIGINAL ARTICLE INFECTIOUS DISEASES

Unexpectedly high prevalence of *Treponema pallidum* infection in the oral cavity of human immunodeficiency virus-infected patients with early syphilis who had engaged in unprotected sex practices

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Abstract

Between 2010 and 2014, we obtained swab specimens to detect *Treponema pallidum*, with PCR assays, from the oral cavities of 240 patients with 267 episodes of syphilis who reported engaging in unprotected sex practices. The detected treponemal DNA was subjected to genotyping. All of the syphilis cases occurred in men who have sex with men (MSM), and 242 (90.6%) occurred in human immunodeficiency virus-infected patients. The stages of syphilis included 38 cases (14.2%) of primary syphilis of the genital region, 76 (28.5%) of secondary syphilis, 21 (7.9%) of primary and secondary syphilis, 125 (46.8%) of early latent syphilis, and seven (2.6%) others. Concurrent oral ulcers were identified in 22 cases (8.2%). Treponemal DNA was identified from the swabs of 113 patients (42.2%), including 15 (68.2%) with oral ulcers. The most common genotype of *T. pallidum* was 14f/f. The presence of oral ulcers was associated with identification of *T. pallidum* in the swab specimens (15/22 (68.2%) vs. 98/245 (40.0%)) ($p = 0.01$). In multivariate analysis, secondary syphilis (adjusted OR 6.79; 95% CI 1.97–23.28) and rapid plasma reagin (RPR) titres of $\geq$1: 32 (adjusted OR 2.23; 95% CI 1.02–4.89) were independently associated with the presence of treponemal DNA in patients without oral ulcers. We conclude that detection of treponemal DNA in the oral cavity with PCR assays is not uncommon in MSM, most of whom reported having unprotected oral sex. Although the presence of oral ulcers is significantly associated with detection of treponemal DNA, treponemal DNA is more likely to be identified in patients without oral ulcers who present with secondary syphilis and RPR titres of $\geq$1: 32.

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Introduction

The incidence of syphilis has been increasing globally in both developed and less developed countries, especially among men who have sex with men (MSM) [1–3]. The increasing incidence of syphilis is associated with a higher risk of transmission of not only human immunodeficiency virus (HIV) but also hepatitis

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viruses [4–7]. The possible mechanisms of increased HIV transmission may be related to disruption of the genital epithelium, recruitment of HIV target cells to the lesion sites, and increased HIV viral load [8].

Although syphilis is caused by infection with *Treponema pallidum* ssp. *pallidum*, the causative pathogen cannot be cultivated *in vitro*. Therefore, microbiological diagnosis of syphilis remains challenging. In clinical practice, diagnosis of syphilis usually depends on detection of *T. pallidum* with dark-field microscopic examination of the mucosal or skin lesions; or serological tests, including non-treponemal tests (rapid plasma reagin (RPR) or Venereal Disease Research Laboratory) and treponemal tests (*T. pallidum* haemagglutination assay, *T. pallidum* particle haemagglutination assay (TPPA), fluorescent treponemal antibody absorption test, treponemal enzyme immunoassay, or chemiluminescence immunoassay) [9,10]. The chemiluminescence immunoassay and enzyme immunoassay have been recommended for confirmation of syphilis by the International Union Against Sexually Transmitted Infection (Europe) [10]. Most recently, a PCR assay has been used to detect *T. pallidum* in various clinical specimens, facilitating investigations of molecular epidemiology and detection of macrolide resistance mutations of *T. pallidum* [11–16].

High rates of PCR detection (60–90%) of *T. pallidum* from the genital, oral mucosal or skin lesions of patients presenting with primary syphilis and secondary syphilis have been reported, which suggests that higher rates of syphilis transmission may occur during oral sex, which may also enhance the transmission of HIV [17,18]. Moreover, infection with syphilis has been found to be associated with oral sex in patients who seldom or never use condoms [19]. Therefore, the oral cavity is an important but easily forgotten portal of entry for sexually transmitted diseases (STDs). Whether *T. pallidum* can be identified in patients with syphilis who do not present with oral mucosal lesions has rarely been systematically studied. In this study, we aimed to investigate the prevalence of *T. pallidum* infection, with the use of PCR assays, in the oral cavities of patients who received a diagnosis of syphilis, and to identify the associated factors.

Materials and methods

Study design and participants

This was a single-centre, prospective survey conducted in infectious diseases clinics between December 2010 and June 2014. During the study period, patients aged ≥20 years who presented to the clinics with the diagnosis of any stage of syphilis, RPR titres of ≥1: 4 and positive TPPA results were enrolled. The clinical information collected included demographics, HIV serostatus, sexual preference, CD4 count and plasma HIV RNA load (in HIV-infected patients), viral hepatitis co-infection, stage of syphilis, treatment of syphilis, and serological response after treatment. HIV-uninfected patients were recruited from a voluntary counselling and testing site for HIV and STDs at the hospital [20]. The stages of syphilis were defined according to the Sexually Transmitted Diseases Treatment Guidelines of the US CDC [21]. The HIV case management programme implemented by Taiwan's CDC to control HIV and STDs requires face-to-face interviews to inquire about the risks for syphilis to be conducted, and counselling to be offered by case managers and treating physicians to those who receive a diagnosis of STD. The study was approved by the Research Ethics Committee of the hospital, and written informed consent was obtained from each patient.

Collection of clinical specimens

All appropriate clinical specimens were collected with the use of swabs (CultureSwab EZ; BD, Franklin Lakes, NJ, USA) before the patients received antibiotic treatment for syphilis. For patients with oral lesions involving the mucosa of the oral cavity (the gingiva, soft palate, hard palate, mouth floor, and tongue) or the lips (Fig. S1A,1B) who were diagnosed as having syphilis of any stage, the swabs were pressed and rolled over the lesions. The exudates expressed on the lesions were absorbed onto the swabs. For syphilis patients without any visible oral or lip mucosal lesions, the swab specimens were collected by rolling the swabs over the upper and lower gingiva, tonsils, hard palate, and soft palate. In addition, concomitant collection of other clinical specimens, including swabs from the genital or anal ulcers in patients with primary syphilis, plasma from patients with secondary syphilis and early latent syphilis, and cerebrospinal fluid from patients with neurosyphilis, was performed; and all of the specimens were subjected to treponemal PCR assays.

Laboratory investigations

Treponemal DNA was extracted from clinical specimens with the Qiagen DNA minikit (Qiagen, Hildens, Germany), according to the manufacturer's protocol. The presence of *T. pallidum* was determined by the amplification of the polymerase I gene (*polA*), as previously described [12,13], with positive and negative controls for *T. pallidum* being included. The detection of macrolide resistance mutations (A2058G or A2059G) in the 23S rRNA gene was performed with PCR–restriction fragment length polymorphism [14,15]. Molecular typing was performed on the basis of three treponemal genes: the number of 60-bp repeats in the acidic repeat protein gene (*arp*); the PCR–restriction fragment length polymorphism patterns of *T. pallidum* repeat genes, including *tprE*, *tprG*, *tprJ*, and *tprC*; and the sequence of a short region (131–215 bp) in *tp0548* [16].

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Plasma HIV RNA load was quantified with the Cobas Amplicor HIV-1 Monitor test (Cobas Amplicor version 1.5; Roche Diagnostics Corporation, Indianapolis, IN, USA), with a lower detection limit of 20 copies/mL, and the CD4 lymphocyte count was determined with FACFlow (BD FACS Calibur, Becton Dickinson, San Jose, CA, USA).

Serological tests for syphilis included the RPR test (Macro-Vue TMRPR Card tests; Becton Dickinson BD Microbiology Systems, Sparks, MD, USA) and the *T. pallidum* particle agglutination test (FTI-SERODIA-TPPA; Fujirebio Taiwan, Taoyuan, Taiwan). According to the national HIV treatment guidelines in Taiwan, testing for RPR titres was performed once every 3–6 months, depending on occurrences of syphilis and treatment. For patients with early syphilis (primary, secondary and early latent syphilis) presenting for treatment, RPR titres were determined every 3 months for 1 year [22]; and for those who had no syphilis or had syphilis that had been treated for >1 year, RPR titres were determined every 6 months, along with determinations of CD4 count and plasma HIV viral load. For HIV-uninfected patients with syphilis who were receiving syphilis treatment, RPR titres and HIV serostatus were determined every 3 months at the voluntary counselling and testing site.

Statistical analysis

All statistical analyses were performed with SPSS version 18.0 (SPSS, Chicago, IL, USA). Categorical variables were compared by the use of Fisher's exact test or the chi-square test. Non-categorical variables were compared by use of the Mann–Whitney *U*-test. Factors with a p-value of $\leq$0.2 or biological significance were included in the multivariate analysis. All comparisons were two-tailed and a p-value of <0.05 was considered to be significant.

Results

During the 4-year study period, 240 patients with 267 episodes of syphilis were enrolled, including 242 episodes (90.6%) occurring in HIV-infected patients and 25 episodes (9.4%) occurring in HIV-uninfected patients. The characteristics of the patients with different stages of syphilis are shown in Table 1. All of the patients were male, and their sexual preference was MSM. Among the 240 HIV-infected patients, 197 (82.1%) of whom were receiving combination antiretroviral therapy when a diagnosis of syphilis was made, the mean CD4 count was 520 cells/mm^3 and the plasma HIV RNA load was 2.21 $\log_{10}$ copies/

TABLE 1. Clinical characteristics of the 240 patients with 267 cases of syphilis

	All cases (*n* = 267)	Primary syphilis (*n* = 38)	Secondary syphilis (*n* = 76)	Primary + secondary syphilis (*n* = 21)	Early latent syphilis (*n* = 125)	Late latent syphilis (*n* = 5)	Neurosyphilis (*n* = 2)
Age (years), mean (SD)	33.1 (7.2)	34.3 (6.9)	30.0 (7.3)	33.4 (7.5)	34.5 (6.7)	32.4 (2.9)	40.5 (0.71)
Male gender, *n* (%)	267 (100)	38 (100)	76 (100)	21 (100)	125 (100)	5 (100)	2 (100)
Sexual preference, *n* (%)							
MSM	267 (100)	38 (100)	76 (100)	21 (100)	125 (100)	5 (100)	2 (100)
Oral lesions[a], *n* (%)							
With oral ulcers	22 (8.2)	17 (44.7)	0 (0)	4 (19.0)	0 (0)	0 (0)	1 (50.0)
Without oral ulcers	245 (91.8)	21 (55.3)	76 (100)	17 (81.0)	125 (100)	5 (100)	1 (50.0)
Oral swab, *n* (%)							
PCR positive	113 (42.3)	13 (34.2)	49 (64.5)	13 (61.9)	35 (28.0)	2 (40.0)	1 (50.0)
PCR negative	154 (57.7)	25 (65.8)	27 (35.5)	8 (38.1)	90 (72.0)	3 (60.0)	1 (50.0)
Oral sex[b], *n* (%)	238 (89.1)	35 (92.1)	67 (88.2)	19 (90.5)	111 (88.8)	5 (100.0)	1 (50.0)
Condom use[c], *n* (%)							
<50%	210 (78.7)	32 (84.2)	60 (78.9)	15 (71.4)	97 (77.6)	5 (100)	0 (0)
>50%	25 (9.4)	3 (7.9)	5 (6.6)	4 (19.0)	12 (9.6)	0 (0)	1 (50.0)
100%	4 (1.5)	0 (0)	2 (2.6)	0 (0)	2 (1.6)	0 (0)	0 (0)
Unknown	29 (10.9)	3 (7.9)	9 (11.8)	2 (9.5)	14 (11.2)	0 (0)	1 (50.0)
HIV infection, *n* (%)	242 (90.6)	32 (84.2)	68 (89.5)	16 (76.2)	120 (96.0)	4 (80.0)	2 (100)
CD4 (cells/mm^3), mean (SD)	520 (255)	429 (181)	498 (240)	464 (225)	570 (269)	613 (331)	90 (104)
$\log_{10}$ HIV RNA (copies/mL), mean (SD)	2.21 (1.39)	2.38 (1.47)	2.35 (1.43)	3.20 (1.52)	1.88 (1.19)	3.02 (1.58)	5.18 (0.72)
On cART	197 (81.4)	26 (81.3)	52 (76.5)	9 (56.2)	107 (89.2)	3 (75.0)	0 (0)
RPR titre, median (IQR)	64 (32–128)	64 (32–256)	128 (64–256)	128 (64–256)	64 (16–128)	32 (8–32)	NA
$\log_2$ RPR titre $\geq$5, *n* (%)	212 (79.4)	32 (84.2)	69 (90.8)	20 (95.2)	86 (68.8)	3 (60.0)	2 (100)
HBsAg, *n* (%)							
Positive	30 (11.2)	3 (7.9)	7 (9.2)	4 (19.0)	15 (0.12)	1 (20.0)	0 (0)
Negative	207 (77.5)	30 (78.9)	59 (77.6)	12 (57.1)	101 (80.8)	3 (60.0)	2 (100)
Unknown	30 (11.2)	5 (13.2)	10 (13.2)	5 (23.8)	9 (7.2)	1 (20.0)	0 (0)
Anti-HCV antibody, *n* (%)							
Positive	21 (7.9)	3 (7.9)	6 (7.9)	1 (4.8)	11 (8.8)	0 (0)	0 (0)
Negative	222 (83.1)	30 (78.9)	61 (80.3)	16 (76.2)	109 (87.2)	4 (80.0)	2 (100)
Unknown	24 (9.0)	5 (13.2)	9 (11.8)	4 (19.0)	5 (4.0)	1 (20.0)	0 (0)
HCV seroconversion[d], *n* (%)	6 (2.2)	0 (0)	1 (1.3)	0 (0)	4 (3.2)	11 (20.0)	0 (0)

cART, combination antiretroviral therapy; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IQR, interquartile range; MSM, men who have sex with men; NA, Not applicable; RPR, rapid plasma reagin; SD, standard deviation.
[a]'Oral lesions' refers to mucosal ulcers on the mucosa of the oral cavity (the gingiva, soft palate, hard palate, mouth floor, and tongue) or the lips suggesting chancres.
[b]'Oral sex' was defined as having had oral sex within 3 months before the diagnosis of syphilis was made.
[c]'Condom use' was defined as the frequency of condom use during sexual activity within 3 months before the diagnosis of syphilis was made.
[d]'HCV seroconversion' was defined as seroconversion of anti-HCV antibody within 12 months after the diagnosis of syphilis was made.

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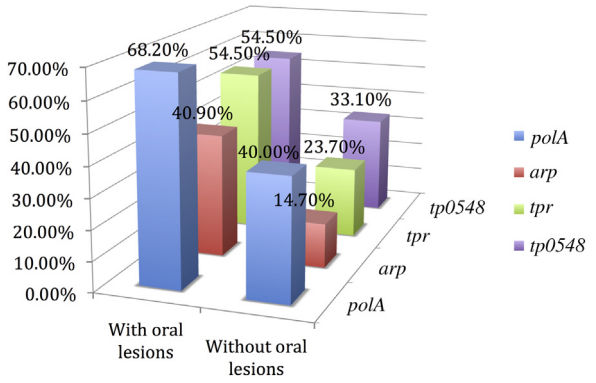


FIG. 1. Yield rates of PCR assay for detection of polymerase A (*polA*), *arp*, *tpr* and *tp0548* genes from oral swabs in patients with or without oral ulcers.

mL (Table 1). None of the 25 HIV-uninfected patients with 25 episodes of syphilis seroconverted for HIV during the follow-up.

Among the 267 episodes of syphilis, oral ulcers consistent with chancres (Fig. S1A, 1B) were found in 22 cases (8.2%). Of 38 cases of primary syphilis only, 17 cases (44.7%) showed concurrent oral ulcers, and treponemal DNA was identified in 13 (34.2%) of the 38 oral swab specimens, all from patients with oral ulcers (76.5%, 13/17). More than 60% (64.5%) of the oral swab specimens from patients with secondary syphilis or patients with both primary and secondary syphilis were positive for *T. pallidum* by PCR assay, regardless of the presence of oral ulcers. Among patients with early latent syphilis and late latent syphilis who had visually intact oral mucosa, *T. pallidum* DNA was detected in 28.0% and 40.0% of the oral swab specimens, respectively. On face-to-face interview, nearly 90% (89.1%) of the patients with any stage of syphilis reported having had oral sex, and only 1.5% of the patients consistently used condoms for either ano-genital sex or oral sex within 3 months before syphilis diagnosis (Table 1).

In the univariate analysis including all patients, we found that younger age (31.4 ± 6.5 years vs. 34.4 ± 7.3 years, p = 0.001), the presence of oral ulcers (15/22 (68.2%) vs. 98/245 (40.0%), p = 0.01), secondary syphilis (43.4% vs. 17.4%, p < 0.001), a higher median RPR titre (128 vs. 64, p = 0.002) and RPR titres of ≥1: 32 (90.3% vs. 71.6%, p < 0.001) were associated with detection of treponemal DNA by PCR assays of the oral swab specimens (data not shown).

The results of univariate and multivariate analyses of factors associated with detection of treponemal DNA by PCR assays of the oral swab specimens in the 245 cases without oral ulcers are shown in Table 2. Secondary syphilis (46.9% vs. 18.4%), a CD4 lymphocyte count of 200–350 cells/mm^3, a higher median RPR titre (128 vs. 32) and an RPR titre of ≥1: 32 (88.8% vs. 70.0%) were found to be associated with detection of treponemal DNA in univariate analysis. In multivariate analysis, we found that presentation with secondary syphilis was statistically significantly associated with detection of treponemal DNA in the oral cavity in those patients who did not present with oral ulcers, with an adjusted OR of 6.79 (95% CI 1.97–23.38).

At screening, 95 of 113 oral swab specimens (84.1%) that were positive for *polA* were also positive for 23S rRNA, and only one strain of *T. pallidum* (1.1%) harboured the A2058G mutation, which confers macrolide resistance. Yield rates of PCR assays used to detect *polA* (screening), *arp*, *tpr* and *tp0548* in the oral swab specimens from patients with or without oral ulcers are shown in Fig. 1. Consistently higher yield rates for detection of *polA* (68.2% vs. 40.0%), *arp* (40.9% vs. 14.7%), *tpr* (54.5% vs. 23.7%) and *tp0548* (54.5% vs. 33.1%) were found in the swab specimens from patients with oral ulcers than in those from patients without oral ulcers.

The full genotypes of *T. pallidum* identified from the oral swab specimens were determined, and the distribution is shown in Fig. 2. Of the 113 amplifiable specimens, 45 (40.0%) were positive for all *tpr*, *arp* and *tp0548* genes, yielding seven full subtypes. Regarding the distribution of genotypes, 14f/f was the most common subtype (28/45, 62.2%), followed by 14b/c (9/45, 20%), 14a/f (3/45, 6.7%), 10b/a (2/45, 4.4%), 13b/a (1/45, 2.2%), 14b/a (1/45, 2.2%), and 14b/f (1/45, 2.2%). Treponemal DNA was concomitantly identified from clinical specimens other than oral swabs in 33 patients, 31 of whom (93.9%) had infections with the strains from other specimens that were of the same genotypes as those strains detected from the oral swabs. Two patients had infections with strains that differed in genotype between oral swabs and other specimens. Both patients were diagnosed as having secondary syphilis, and the specimens for detection of treponemal DNA were oral swabs and plasma. In one patient, the molecular subtype of the strain from the oral swab specimen (without oral ulcers) was 14/b/c, and the molecular subtype of the strain from the plasma specimen was a/f. In the other patient, the molecular subtype of the strain from the oral swab was /j/, and the molecular subtype of the strain from plasma was b/c.

Discussion

In this study, we found that younger age (31.4 ± 6.5 years vs. 34.4 ± 7.3 years), oral ulcers (15/22 (68.2%) vs. 98/245 (40.0%)), secondary syphilis (43.4% vs. 17.4%), a higher median RPR titre (128 vs. 64) and an RPR titre of ≥1: 32 (90.3% vs. 71.6%) were statistically significantly associated with detection of treponemal DNA, with PCR assays, from the oral swab specimens in patients with syphilis. The rate of detection of treponemal DNA in the oral swab specimens collected from

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TABLE 2. Univariate and multivariate analysis of the factors associated with positive PCR results for *Treponema pallidum* in patients without oral ulcers

	Univariate analysis			Multivariate analysis		
	Oral swab with positive PCR result (n = 98)	Oral swab with negative PCR result (n = 147)	p	Adjusted OR	95% CI	p
Age (years), mean (SD)	31.2 (6.6)	34.3 (7.4)	0.11	0.96	0.92–1.00	0.08
Male gender, *n* (%)	98 (100)	147 (100)	—	—	—	—
Sexual preference, *n* (%)						
MSM	98 (100)	147 (100)	—	—	—	—
Syphilis stage, *n* (%)			<0.001			
Primary	4 (4.1)	17 (11.6)		1	—	—
Secondary	46 (46.9)	27 (18.4)		6.79	1.97–23.38	0.002
Other stages	48 (49.0)	103 (70.0)		1.83	0.56–5.97	0.32
Oral sex, *n* (%)	91 (92.9)	129 (87.8)	0.65	2.87	0.21–39.56	0.43
Condom use, *n* (%)			0.39			
<50%	79 (80.6)	115 (78.2)		1	—	—
>50%	9 (9.2)	13 (8.8)		0.15	0.01–2.17	0.16
100%	3 (3.1)	1 (0.7)		0.16	0.01–1.97	0.15
Unknown	6 (6.1)	18 (12.2)		—	—	—
HIV infection, *n* (%)	86 (87.8)	135 (91.8)	0.38	—	—	—
CD4 ≤200	4 (4.7)	6 (4.4)	1	1	—	—
CD4 >200, CD4 ≤350	22 (22.4)	19 (14.1)	0.025	1.51	0.32–7.11	0.6
CD4 >350	60 (61.2)	110 (81.5)	0.05	0.77	0.19–3.14	0.72
PVL <20 copies/mL, *n* (%)	49 (50.0)	72 (53.3)	0.58	1.57	0.82–2.99	0.17
On cART, *n* (%)	72 (73.5)	111 (82.2)	0.86	—	—	—
RPR titre, median (IQR)	128 (64–256)	32 (16–64)	<0.001	—	—	—
RPR titre ≥1: 32	87 (88.8)	103 (70.0)	0.001	2.23	1.02–4.89	0.045
HBsAg, *n* (%)			0.84			
Positive	11 (11.2)	19 (12.9)		0.81	0.32–2.05	0.66
Negative	73 (74.5)	113 (76.9)		1	—	—
Unknown	14 (14.3)	15 (10.2)		—	—	—
Anti-HCV antibody, *n* (%)			1			
Positive	8 (8.2)	12 (8.2)		1.01	0.34–3.03	0.99
Negative	78 (79.6)	124 (84.4)		1	—	—
Unknown	12 (12.2)	11 (7.5)		—	—	—
HCV seroconversion, *n* (%)	2 (2.0)	4 (2.7)	1	1.06	0.14–7.74	0.96

cART, combined antiretroviral therapy; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IQR, interquartile range; MSM, men who have sex with men; PVL, plasma HIV RNA load; RPR, rapid plasma reagin; SD, standard deviation.

patients without oral ulcers was strikingly high (40.0%), and was associated with secondary syphilis in multivariate analysis.

Recently, the incidence rates of primary and secondary syphilis have been increasing, especially among MSM and post-adolescent individuals (aged 20–24 years) [1]. In addition, a high level of association between syphilis and HIV acquisition has already been noted among MSM worldwide [20,23–26]. On most of these occasions, unprotected sexual intercourse has been shown to increase the risks of syphilis and HIV transmission [20,26]. Although information, education and communication to promote safe practices have been provided

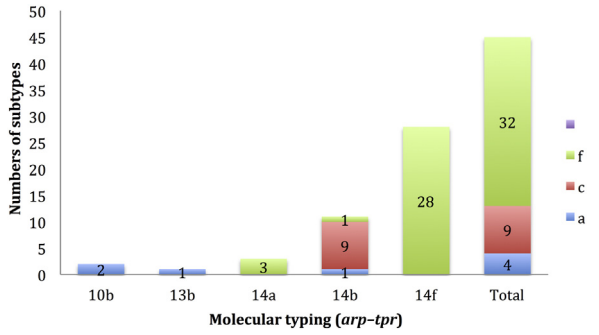


FIG. 2. Distribution of genotypes of *Treponema pallidum* strains.

[1], the message of safe sex may be received and interpreted in the context of genital-to-genital or genital-to-anus sex. Although the detection of *T. pallidum* DNA from the oral mucosa in our study does not translate to the presence of viable, infectious *T. pallidum*, our finding of a high percentage of detectable treponemal DNA (40.0%) in patients with visually intact oral mucosa implies that the oral cavity could possibly serve as a neglected portal of entry for *T. pallidum*, and that oral sex could be a neglected transmission route. The same scenario of transmission of syphilis through unprotected oral sex has been observed for the transmission of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* from the oropharynx to the urethra, or vice versa, and the oropharynx could be a reservoir of sexually transmitted pathogens in asymptomatic MSM [27]. Thus, the high prevalence of detectable *T. pallidum* in the oral cavities of asymptomatic patients in our study should serve as a reminder to those who provide treatment and counselling to the patients, as well as the individuals who practise unprotected oral sex, with regard to ongoing syphilis transmission.

Our study is the first to systematically perform PCR assays for detection of *T. pallidum* infection in the oral cavities of patients with syphilis, regardless of the presence of oral ulcers

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when syphilis was diagnosed. However, there are several limitations to our study, and the results should be interpreted with caution. First, the rate of detection of treponemal DNA could be underestimated in patients without oral ulcers, owing to limitations of the current detection methodology. However, there were no reference rates in previously published studies, and the frequency of the presence of treponemal DNA in the oral cavity in our study (40.0%) is substantial. Second, oral ulcers are easier to identify, and oral mucosa patches may be present in patients with secondary syphilis; however, the oral mucosa patches of syphilis may be too subtle and be overlooked by the treating physicians, leading to misclassification of syphilis stage. Third, the stages of primary and secondary syphilis are defined according to the presenting symptoms in clinical practice. For the patients in whom syphilis progresses from the primary stage to the secondary stage but without detectable residual chancres or mucocutaneous lesions, there are no good surrogate markers to differentiate between the two stages, and the clinical stage may also be misclassified.

In conclusion, detection of treponemal DNA in the oral cavity with PCR assays is not uncommon in MSM who practise unprotected oral sex, even in the syphilitic individuals with apparently intact oral mucosa. Barrier protection to prevent transmission of STDs at all points of sexual contact, including oral–genital or oral–anal contacts, cannot be overemphasized.

Transparency declaration

The authors state that they have no conflicts of interest.

Author contributions

C.-J. Yang, S.-Y. Chang, C.-C. Hung and S.-C. Chang conceived and designed the experiments. C.-J. Yang, S.-Y. Chang, B.-R. Wu, S.-P. Yang, W.-C. Liu, P.-Y. Wu, J.-Y. Zhang and Y.-Z. Lou performed the experiments. C.-J. Yang, S.-Y. Chang and C.-C. Hung analyzed the data. C.-J. Yang, S.-Y. Chang, C.-C. Hung and S.-C. Chang wrote the paper.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2015.04.018

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Evaluation of the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry Bruker Biotyper for identification of *Penicillium marneffei*, *Paecilomyces* species, *Fusarium solani*, *Rhizopus* species, and *Pseudallescheria boydii*

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We evaluated the performance of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), the MALDI Bruker Biotyper system (microflex LT; Bruker Daltonik GmbH, Bremen, Germany), on the identification of 50 isolates of clinically encountered molds, including *Penicillium marneffei* (n = 28), *Paecilomyces* species (n = 12), *Fusarium solani* (n = 6), *Rhizopus* species (n = 3), and *Pseudallescheria boydii* (n = 1). The isolates were identified to species levels by sequence analysis of the internal transcribed spacer (ITS) regions using primers ITS1 and ITS4. None of the 28 genetically well characterized isolates of *P. marneffei* were identified as *P. marneffei* by MALDI-TOF MS, because *P. marneffei* was not present in either Bruker general library (DB 5627) or Bruker filamentous fungi library V1.0. However, the rate of accurate identification as *P. marneffei* (score value $\geq$ 2.000) was 85.7% based on newly created database from one *P. marneffei* strain (NTUH-3370) by MALDI Biotyper system. Sequencing analysis of these 22 non-*P. marneffei* isolates of molds revealed seven *Paecilomyces variotii*, six *F. solani*, four *Paecilomyces lilacinus,* and one each of *Paecilomyces sinensis*, *Rhizopus arrhizus, R. oryzae, R. microspores,* and *P. boydii*. Although all the seven *P. variotii* isolates, four of the six *F. solani*, two of the four *P. lilacinus,* and two of the three isolates of *Rhizopus* species, and the *P. boydii* isolate had concordant identification results between MALDI-TOF MS and sequencing analysis, the score values of these isolates were all of <1.700. This study indicated that the MALDI Bruker Biotyper is ineffective for identifying *P. marneffei* and other unusual molds because of the current database limitations. Therefore, it is necessary to continuously update the MALDI-TOF MS databases.

Keywords: matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, *Penicillium marneffei*, *Paecilomyces species*, *Fusarium solani*, *Rhizopus* species, *Pseudallescheria boydii*

Introduction

Although invasive aspergillosis represents the most common opportunistic mold infection, less commonly encountered molds, such as *Fusarium Paecilomyces,* and *Zygomycetes* have increasingly been reported to cause invasive mold infections (Schalk et al., 2006; Malani and Kauffman, 2007; Hsiue et al., 2010b; Bassiri-Jahromi, 2014). Infections caused by non-*Aspergillus* molds are associated with a substantially worse outcome than invasive aspergillosis (Schalk et al., 2006; Malani and Kauffman, 2007; Hsiue et al., 2010b; Bassiri-Jahromi, 2014), which is most likely attributed both to the significant immunodeficient state of these patients and the intrinsically less susceptibility of these organisms to antifungal agents (Schalk et al., 2006; Malani and Kauffman, 2007; Hsiue et al., 2010b; Bassiri-Jahromi, 2014). *Penicillium marneffei* has become a well-recognized pathogen in humans and is an important emerging public health threat (Duong, 1996; Hsueh et al., 2000; Hung et al., 2012; Lee et al., 2012). Infection caused by this organism is frequently disseminated and is progressive in nature among immunocompromised hosts, particularly among patients with impaired cell-mediated immunity and human immunodeficiency virus (HIV) infection (Duong, 1996; Hsueh et al., 2000; Hung et al., 2012; Lee et al., 2012). A history of recent travel in Southeast Asia, the southern part of China, Thailand, Vietnam, or Indonesia is common among patients with *P. marneffei* infection outside Asia (Supparatpinyo et al., 1994; Larsson et al., 2012; Hu et al., 2013). Due to the life-threatening entity of infections caused by these non-*Aspergillus* molds, prompt diagnosis and appropriate antifungal treatments are crucial (Duong, 1996; Schalk et al., 2006; Malani and Kauffman, 2007; Hsiue et al., 2010b; Bassiri-Jahromi, 2014).

In clinical microbiology laboratories, mold identification largely based on the macroscopic and microscopic observation of colonies grown on mycological media culture of or direct examination of the organisms in the histopathological sections (Hsiue et al., 2010a; Cassagne et al., 2011; Chalupová et al., 2014). Adequate phenotypic identification of molds, especially non-*Aspergillus* molds, requires highly skilled mycologists, who are seldom found in clinical mycology laboratories (Cassagne et al., 2011). Furthermore, these methods are time-consuming and sometimes unreliable (Hsiue et al., 2010a; Cassagne et al., 2011; Chalupová et al., 2014). There have been a number of molecular methods to identify non-*Aspergillus* molds from various clinical specimens or directly identify these organisms in the infected tissue, however, these detection methods are usually available only in research laboratories (Hsiue et al., 2010a; Zhang et al., 2011).

The matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), which is now widely used in clinical microbiology laboratories, can rapidly and accurately identify different species of bacteria and fungi (Murray, 2010; Alanio et al., 2011; Putignani et al., 2011; Nenoff et al., 2013; Chao et al., 2014; Ling et al., 2014). However, species identification of these non-*Aspergillus* molds, particularly *P. marneffei,* using this method has seldom been reported (Marinach-Patrice et al., 2009; Santos et al., 2009; De Carolis et al., 2012; Del Chierico et al., 2012; Barker et al., 2014; Triest et al., 2015). In this study, we evaluated the performance of MALDI-TOF MS for the identification of clinical isolates of non-*Aspergillus* molds, especially *P. marneffei* that were confirmed to the species levels by using sequencing analysis methods.

Materials and Methods

Fungal Isolates

Twenty-eight preserved non-duplicate isolates of *P. marneffei* were recovered from various clinical specimens from 28 patients who were treated at National Taiwan University Hospital (NTUH), a 2900-bed teaching hospital in northern Taiwan, from January 2000 through December 2012 (**Table 1**). Among these patients, 18 had AIDS and three (two in Thailand and one in Myanmar) acquired *P. marneffei* infection outside Taiwan. Clinical specimens for isolation were inoculated onto Sabouraud dextrose agar (Becton Dickinson Microbiology Systems, Sparks, MD, USA) and incubated at 25°C in ambient air. These isolates were identified as *P. marneffei* using conventional methods.

The other 22 isolates of non- *P. marneffei* isolates of molds were recovered from various clinical specimens of 22 patients who were treated at the hospital from 2010 to 2012 (Hsiue et al., 2010b). Among these isolates, six were initially identified by phenotypic methods as *F. solani,* four were *Paecilomyces lilacinus,* five were *Paecilomyces variotii,* three were *Rhizopus* sp., two were *Talaromyces spectabilis,* and one each of *Paecilomyces sinensis* and *Pseudallescheria boydii.*

Identification by Gene Sequencing Analysis

The isolates were further identified by sequence comparison analysis of the internal transcribed spacer (ITS) regions using primers ITS1 and ITS4 as previously described (Hsiue et al., 2010a,b).

Identification by MALDI-TOF MS

For performance of MALDI-TOF MS system, the MALDI Biotyper system (microflex LT; Bruker Daltonik GmbH, Bremen, Germany) was used (Chao et al., 2014). A loopful of mycelial colonies from Sabouraud dextrose agar medium was transferred to into a 1.5-ml microtube containing 300 µl of pure water and then added 900 µl of pure ethanol. The suspension was pelleted after centrifugation at 12,000 rpm for 2 min. The supernatant was discarded and 1 ml of 75% ethanol was added, centrifuged after 12,000 rpm for 2 min, and the supernatant was discarded. The pellet was dried for 1 h. The sample was then subjected to a standard ethanol–formic acid extraction procedure. A volume of 1.0 µl of the supernatant was applied to a 96-spot polished steel target (Bruker Daltonik GmbH, Bremen, Germany) plate and dried. A saturated solution of 1.0 µl of MALDI matrix (HCCA; Bruker Daltonik) was applied to each sample and dried. Measurements were performed with Bruker Microflex™ LT MALDI-TOF MS (Bruker Daltonik GmbH)

TABLE 1 | Comparison of the Bruker Biotyper MALDI TOF MS system with that of molecular method in identifying 28 isolates of *Penicillium marneffei*.

No. of Isolates (year of isolation)	Country of acquisition	AIDS	Source of isolates	Identification by sequencing internal transcribed spacer (ITS) region			Identification by MALDI biotyper with preexisting database		Cluster[b]
				Species	Accession no.	Identity (%)	Species	Score value[a]	
1 (2000)	Myanmar	–	Skin	*P. marneffei*	AB363755.1	100	*Penicillium funiculosum*	1.290	II
2 (2000)	Taiwan	–	Pleural effusion	*P. marneffei*	AB363755.1	100	*Microsporum canis*	1.243	-
3 (2000)	Taiwan	–	Spine	*P. marneffei*	AB363755.1	100	*Penicillium funiculosum*	1.468	I
4 (2001)	Taiwan	+	Lung	*P. marneffei*	AB363755.1	100	*Penicillium funiculosum*	1.435	I
5 (2001)	Taiwan	+	Blood	*P. marneffei*	AB363755.1	100	*Penicillium purpurogenum*	1.078	III
6 (2001)	Taiwan	–	Blood	*P. marneffei*	AB363755.1	100	*Penicillium rugulosum*	1.365	III
7 (2001)	Taiwan	+	Middle ear fluid	*P. marneffei*	AB363755.1	100	*Penicillium funiculosum*	1.524	II
8 (2001)	Taiwan	+	Blood	*P. marneffei*	AB363755.1	100	*Penicillium funiculosum*	1.141	III
9 (2002)	Taiwan	–	Blood	*P. marneffei*	AB363755.1	100	*M. canis*	1.280	I
10 (2003)	Taiwan	+	Blood	*P. marneffei*	AB363755.1	100	*M. canis*	1.124	I
11 (2003)	Taiwan	+	Blood	*P. marneffei*	AB363755.1	100	*M. canis*	1.159	I
12 (2004)	Taiwan	+	Blood	*P. marneffei*	AB363755.1	100	*Penicillium funiculosum*	1.013	II
13 (2004)	Taiwan	+	Blood	*P. marneffei*	AB363755.1	100	*Penicillium rugulosum*	1.463	III
14 (2006)	Taiwan	+	Blood	*P. marneffei*	AB363755.1	100	*Penicillium funiculosum*	1.511	IV
15 (2006)	Taiwan	+	Blood	*P. marneffei*	AB363755.1	100	*Penicillium funiculosum*	1.244	IV
16 (2006)	Taiwan	+	Blood	*P. marneffei*	AB363755.1	100	*Penicillium funiculosum*	1.548	III
17 (2007)	Thailand	+	Blood	*P. marneffei*	AB363755.1	100	*Aspergillus candidus*	1.119	I
18 (2007)	Thailand	+	Blood	*P. marneffei*	AB363755.1	100	*Penicillium funiculosum*	1.353	II
19 (2008)	Taiwan	–	Pleural effusion	*P. marneffei*	AB363755.1	100	*Penicillium funiculosum*	1.472	I
20 (2008)	Taiwan	+	Pleural effusion	*P. marneffei*	AB363755.1	100	*Penicillium rugulosum*	1.492	III
21 (2008)	Taiwan	–	Lung	*P. marneffei*	AB363755.1	100	*Penicillium funiculosum*	1.235	II
22 (2008)	Taiwan	–	Lymph node	*P. marneffei*	AB363755.1	100	*Penicillium funiculosum*	1.401	I
23 (2010)	Taiwan	+	Lymph node	*P. marneffei*	AB363755.1	99.6	*Penicillium funiculosum*	1.448	III
24 (2010)	Taiwan	+	Skin mass	*P. marneffei*	AB363755.1	100	*Penicillium rugulosum*	1.413	III
25 (2010)	Taiwan	+	Sputum	*P. marneffei*	AB363755.1	100	*Penicillium funiculosum*	1.304	I
26 (2011)	Taiwan	+	Pleural effusion	*P. marneffei*	AB363755.1	100	*Penicillium funiculosum*	1.614	I
27 (2012)	Taiwan	–	Lung	*P. marneffei*	AB363755.1	100	*Penicillium funiculosum*	1.399	I
28 (2012)	Taiwan	–	Blood	*P. marneffei*	AB363755.1	100	*Trichophyton rubrum*	1.281	III

[a]*All were unreliable identification.*

[b]*Principal component analysis (PCA) dendrogram generated by MALDI Biotyper mass spectra for 28 genetically well characterized isolates of Penicilliun marneffei. The dendrogram of the 28 isolates shows four cluster groups (I, II, III, and IV) with a default critical distance level of 850.*

using FlexControl™ software with CompassFlex Series version 1.3 software and a 60 Hz nitrogen laser (337 nm wavelength). Spectra were collected in the linear positive mode in a mass range covering 1,960–20,132 m/z. Spectra ranging from the mass-to-charge ratio (m/z) 2,000–20,000 were analyzed using MALDI Biotyper automation control and the Bruker Biotyper 3.1 software and Bruker general library (DB 5627) and Bruker filamentous fungi library V1.0. (Bruker Daltonics). Identification scores of ≥2.000 indicated species-level identification, scores of 1.700–1.999 indicated genus-level identification, and scores of <1.700 indicated no identification (Chao et al., 2014). The criteria of identification using standard cut-off value of 2.0 and a lowered cut-off value of 1.4 were also evaluated (Triest et al., 2015).

The principal component analysis (PCA) dendrogram generated by MALDI Biotyper mass spectra obtained from the MALDI Biotyper data of the 28 *P. marneffei* isolates was also performed (Chen et al., 2014).

Results

Fungal Isolates

After 3 days of incubation, the *P. marneffei* isolates grew as mold, with a soluble red pigment diffusing into the agar. The reverse side was red or pink. When seen through a microscope, the isolates had typical features of *Penicillium* species. Colonies were sub-cultured onto brain-heart infusion agar slants (Becton Dickinson Microbiology Systems) and were incubated at 37°C for 3 days to yield the yeast phase. *P. marneffei* yeast cells appeared tubular or oblong, and some had a cross-septum (Hsueh et al., 2000). All the 28 isolates were confirmed as *P. marneffei* (identity 99.6–100%) with accession number of AB363755.1 (**Table 1**).

Among these 22 non-*P. marneffei* isolates of molds, using sequencing analysis, seven were *P. variotii*, six were confirmed as *F. solani*, four were *P. lilacinus*, and one each of *P. sinensis*, *Rhizopus arrhizus*, *R. oryzae*, *R. microspores*, and *P. boydii* (**Table 2**).

Identification by MALDI-TOF for *P. marneffei*

None of the 28 isolates were identified as *P. marneffei* by MALDI biotyper system, because *P. marneffei* was not present in either Bruker general library (DB 5627) or Bruker filamentous fungi library V1.0 (**Table 1**). There are also no reliable identification results to genus level for all the 28 genetically well characterized *P. marneffei* isolates.

The PCA dendrogram generated by MALDI Biotyper mass spectra obtained from the MALDI Biotyper data of the 28 *P. marneffei* isolates is shown in **Figure 1**. Four cluster groups (I, II, III, and IV) with a default critical distance level of 850 were identified in 27 of the 28 isolates (**Figure 1**; **Table 1**).

Database Creation by MALDI-TOF MS for *P. marneffei*

Because *P. marneffei* was not listed in MALDI Biotyper database, four isolates, NTUH-1124 (Isolate 4), NTUH-3370 (Isolate 8), NTUH-7204 (Isolate 12), and NTUH-9736 (Isolate 15) randomly selected from each cluster (I, III, II, and IV, respectively) of the 28 isolates of *P. marneffei* were subjected for database creation for *P. marneffei*. The eluent of each of the four well-prepared isolates was applied for eight spots on MALDI target. Each spot was performed data acquisition for four times. A total of 128 spectra were generated. After evaluation by signal resolution and mass accuracy of the 128 spectra, 27–31 of the 32 spectra from each of the four isolates (27 spectra in NTUH-1124, 29 in NTUH-3370, 28 in NTUH-7204, and 31 in NTUH-9736) were selected for MSP (Main Spectra Projection; database entrance) creation (**Table 2**). The MSP can be created by MALDI Biotyper (Bruker Daltonics) software. We then aimed at validate whether the database generated by the four strains could be used for identification of *P. marneffei*. The database was blindly tested with the 28 *P. marneffei* isolates.

Verification of MALDI-TOF New Database of *P. marneffei*

The best identification score values was found according database created by NTUH-3370: 23 (82.1%) were identified

TABLE 2 | Comparison of identification results by the MALDI Biotyper system with those of molecular method in identifying 22 non-*P. marneffei* isolates of molds.

Species identified by ITS sequencing analysis			Species identification by MALDI Biotyper with preexisting database	
Isolate no. (source)	**Identity (%)**	**Accession number**	**MALDI-TOF results (best match)**	**Score values**
***Fusarium solani* (*n* = 6)**				
1. (Eye)	100	EU314957	*F. solani*	1.622
2. (Tissue)	99	FJ426390	*M. persicolor*	1.046
3. (Eye)	98	AM412600	*F. solani*	1.392
4. (Eye)	98	AM412600	*F. solani*	1.646
5. (Urine)	98	EU314965	*F. solani*	1.186
6. (Skin pus)	99	EU314957	*F. solani*	2.01
***Paecilomyces lilacinus* (*n* = 4)**				
1. (Eye)	99	EU306174	*P. lilacinus*	1.061
2. (Tissue)	99	EU306174	*A. ochraceus*	1.004
3. (Eye)	99	AY213668	*M. gypseum*	0.98
4. (Tissue)	99	AY213668	*P. lilacinus*	0.99
***Paecilomyces variotii* (*n* = 7)**				
1. (Bronchial washing)	98	AY373941	*P. varioti*	1.514
2. (Tissue)	98	EU272527	*P. varioti*	1.251
3. (Sputum)	98	AY904061	*P. varioti*	1.651
4. (Blood)	98	AY373941	*P. varioti*	1.49
5. (Nail biopsy)	98	AY373941	*P. varioti*	1.423
6. (Bronchial washing)	99	EU037066	*P. varioti*	1.112
7. (Necrotic tissue)	100	EU037066	*P. varioti*	1.317
***Paecilomyces sinensis* (*n* = 1)**				
1. (Blood)	99	EU272527	*F. culmorum*	1.14
***Rhizopus arrhizus* (*n* = 1)**				
1. (Skin pus)	99	AF543520	*Rhizopus oryzae*	1.395
***Rhizopus oryzae* (*n* = 1)**				
1. (Bronchial washing)	99	AB109758	*R. oryzae*	1.231
***Rhizopus microspores* (*n* = 1)**				
1. (Pleural effusion)	100	EF151442	*R. microsporus*	1.562
***Pseudallescheria boydii* (*n* = 1)**				
1. (Sputum)	99	EF639871	*P. boydii*	1.163

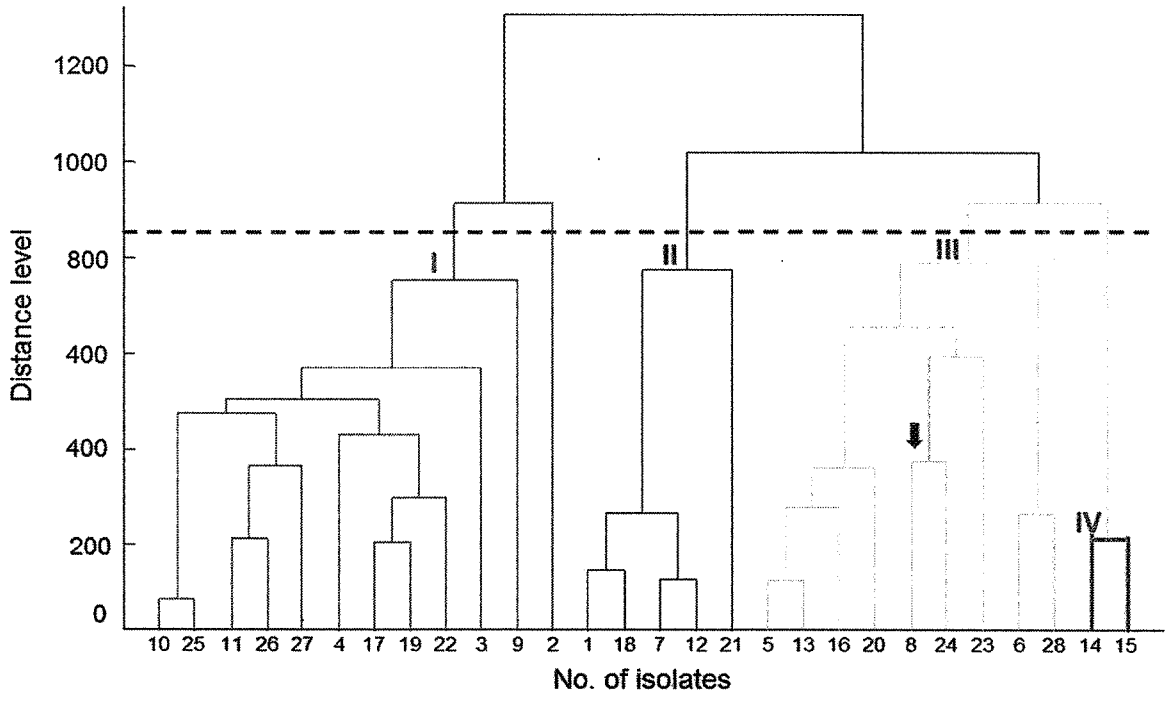


FIGURE 1 | Principal component analysis (PCA) dendrogram generated by MALDI Biotyper mass spectra for 28 genetically well characterized isolates of *Penicillium marneffei*. The arrow indicates the NTUH-3370 isolate. The dendrogram obtained from the MALDI Biotyper data of 25 isolates shows four cluster groups (I, II, III, and IV) with a default critical distance level of 850.

as *P. marneffei* with identification score values of ≥2.000 (2.050–2.289) and the remaining five isolates (17.9%) with score values of 1.809–1.986 (**Table 3**). Twenty, 22, and 13 isolates were identified as *P. marneffei* with score values of ≥2.000 according to MSP made by NTUH-1124, NTUH-7204, and NTUH-9736, respectively. All except one (isolates NTUH-3937 by MSP made by NTUH-7204, score value of 1.525) the remaining isolates possessed identification score values of >1.700 based on MSP made by NTUH-1124, NTUH-7204, and NTUH-9736 (**Table 3**). These characteristic MALDI Biotyper spectra of NTUH-3370 isolate are shown in **Figure 2**.

Identification by MALDI-TOF for non-*P. marneffei* Isolates

Among these 22 non-*P. marneffei* isolates of molds, only one isolate of *F. solani* could be correctly identified by MALDI-TOF MS with score value of ≥2.0 (**Table 2**). Although all the seven *P. variotii* isolates, four of the six *F. solani*, two of the four *P. lilacinus*, and two of the three isolates of *Rhizopus* species, and the *P. boydii* isolate had concordant identification results between MALDI-TOF MS and sequencing analysis, all the score values of these isolates were of <1.700 (**Table 2**). Four of the seven *P. variotii* isolates, three of the six *F. solani*, the *R. microspores* isolate, and none of other molds tested could be correctly identified with lowering the cut-off value of 1.4. These characteristic MALDI Biotyper spectra of non-*P. marneffei* isolates are shown in **Figure 3**.

Discussion

MALDI-TOF MS is a newly developed diagnostic tool that is increasingly being employed to rapidly and accurately identify clinical relevant pathogenic yeasts and molds, including *Aspergillus*, *Fusarium*, *Zygomyces*, *Paecilomyces*, dematophytes, and other molds (16–27, Marinach-Patrice et al., 2009; Santos et al., 2009; Murray, 2010; Alanio et al., 2011; Putignani et al., 2011; De Carolis et al., 2012; Del Chierico et al., 2012; Nenoff et al., 2013; Barker et al., 2014; Chao et al., 2014; Ling et al., 2014; Triest et al., 2015). However, the performance of MALDI-TOF for identification of unusual or several endemic molds depends on the availability of the mold species in the database.

A study conducted by De Carolis et al. (2012) they evaluated the preexisting database of MALDI-TOF MS (MALDI Biotyper software 2.0) using 103 clinical mold isolates belonging to the 55 most clinically relevant species of *Aspergillus* (33 species), *Fusarium* (12 species), and Mucorales (10 species). Multilocus sequence analysis was used as a reference method for species designation. Excluding nine isolates that belong to the fungal species not included in our reference database, 91 (96.8%) of 94 isolates were identified by MALDI-TOF MS to the species level, in agreement with the results of the reference method and the three isolates were identified to the genus level. Recently, Triest et al. (2015) evaluated the performance of MALDI-TOF MS (MALDI Biotyper) for 268 genetically documented (multilocus sequence analysis)

TABLE 3 | Identification results of the 28 isolates of *P. marneffei* by MALDI Biotyper based on four newly created databases for *P. marneffei*.

No. of Isolates (strain designation)	Identification and score values by MALDI Biotyper with newly created database by indicated strains (designation of cluster)				
	Species	Score values[a]			
		NTUH-3370	NTUH-1124	NTUH-7204	NTUH-9736
1. (NTUH-8873)	*P. marneffei*	**1.986**	**1.844**	2.015	**1.900**
2. (NTUH-4566)	*P. marneffei*	2.050	2.029	2.011	**1.931**
3. (NTUH-4779)	*P. marneffei*	2.240	2.157	2.233	2.161
4. (NTUH-1124)	*P. marneffei*	2.464	2.790	2.545	2.313
5. (NTUH-2875)	*P. marneffei*	2.250	2.238	2.147	**1.986**
6. (NTUH-1286)	*P. marneffei*	2.162	2.113	2.266	2.163
7. (NTUH-1710)	*P. marneffei*	2.113	**1.941**	**1.995**	**1.897**
8. (NTUH-3370)	*P. marneffei*	2.770	2.481	2.491	2.265
9. (NTUH-1312)	*P. marneffei*	2.183	2.179	2.271	2.010
10. (NTUH-3937)	*P. marneffei*	**1.809**	**1.857**	1.525	**1.821**
11. (NTUH-6735)	*P. marneffei*	2.076	2.139	2.146	**1.999**
12. (NTUH-7204)	*P. marneffei*	2.559	2.467	2.831	2.451
13. (NTUH-9258)	*P. marneffei*	2.090	**1.963**	2.092	**1.954**
14. (NTUH-0594)	*P. marneffei*	2.100	2.046	2.132	2.046
15. (NTUH-9736)	*P. marneffei*	2.339	2.358	2.442	2.791
16. (NTUH-7483)	*P. marneffei*	2.289	2.089	2.114	**1.918**
17. (NTUH-7342)	*P. marneffei*	**1.860**	**1.982**	1.995	**1.961**
18. (NTUH-7732)	*P. marneffei*	2.224	2.209	2.191	2.006
19. (NTUH-1294)	*P. marneffei*	**1.964**	2.060	2.045	2.059
20. (NTUH-2810)	*P. marneffei*	2.066	**1.940**	2.077	**1.987**
21. (NTUH-0524)	*P. marneffei*	2.016	**1.970**	2.028	**1.914**
22. (NTUH-3286)	*P. marneffei*	**1.880**	**1.975**	**1.930**	**1.942**
23. (NTUH-7870)	*P. marneffei*	2.233	2.004	2.000	**1.977**
24. (NTUH-5723)	*P. marneffei*	2.268	2.127	2.143	2.123
25. (NTUH-1440)	*P. marneffei*	2.061	2.057	**1.903**	**1.909**
26. (NTUH-1934)	*P. marneffei*	2.064	2.056	2.021	2.086
27. (NTUH-0361)	*P. marneffei*	2.083	2.045	**1.996**	**1.834**
28. (NTUH-5587)	*P. marneffei*	2.102	2.116	2.247	2.186

[a]*Score values of 1.700–1.999 are shown in boldface and that of <1.700 is underlined.*

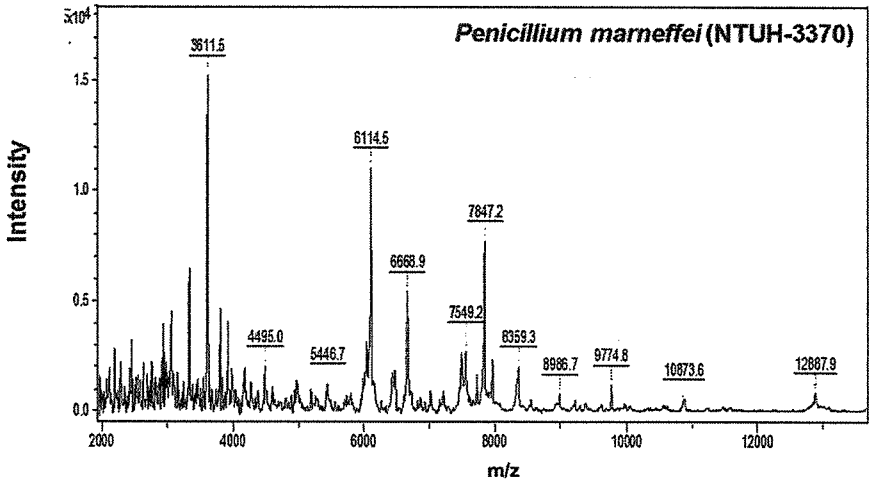


FIGURE 2 | Spectra of *Penicillium marneffei* (NTUH-3370) generated by the MALDI-TOF Bruker Biotyper. The absolute intensities of the ions are shown on the *y* axis, and the masses (*m/z*) of the ions are shown on the *x* axis. The *m/z* values represent the mass-to-charge ratio.

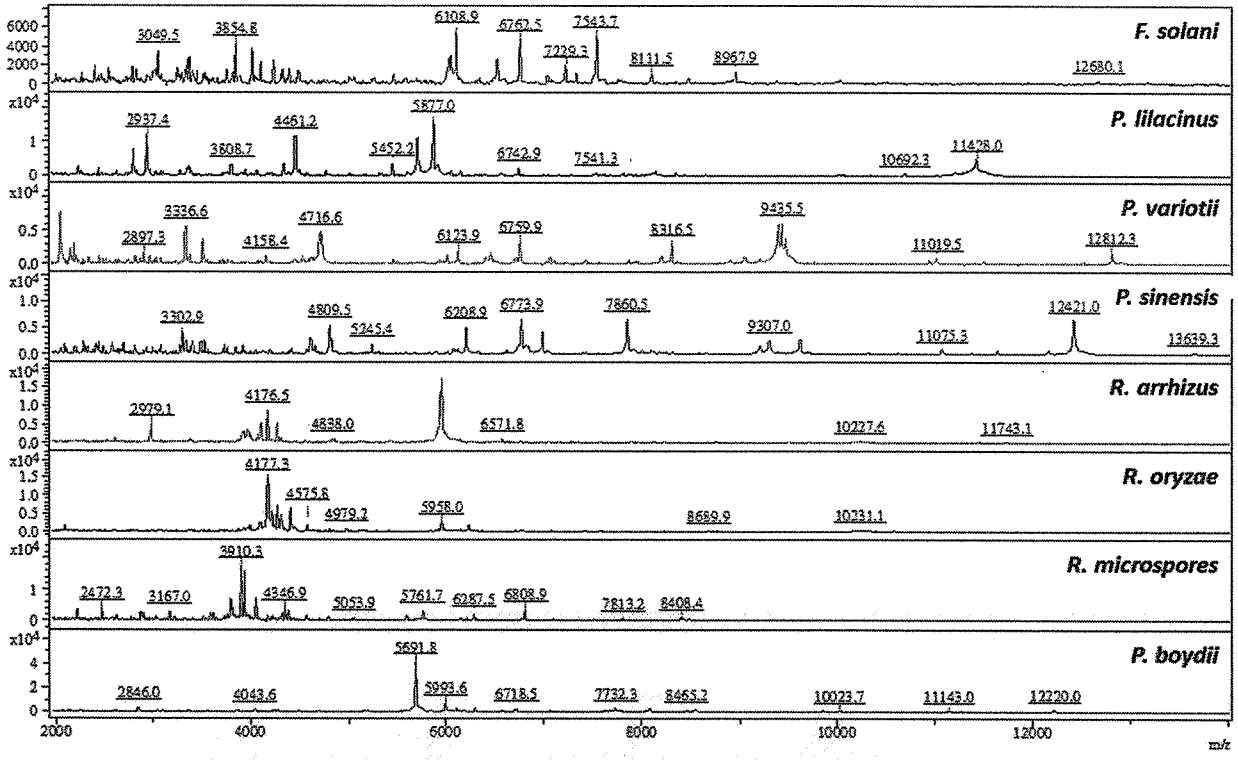


FIGURE 3 | Spectra of *Paecilomyces variotii*, *Fusarium solani*, *Paecilomyces lilacinus*, *Paecilomyces sinensis*, *Rhizopus arrhizus*, *Rhizopus oryzae*, *Rhizopus microspores*, and *Pseudallescheria boydii* generated by the MALDI-TOF Bruker Biotyper. The absolute intensities of the ions are shown on the *y* axis, and the masses (*m/z*) of the ions are shown on the *x* axis. The *m/z* values represent the mass-to-charge ratio.

Fusarium species (19 species) and found that 82,8% of the identifications were correct up to species level according to well-defined identification criteria of score value of 2,0 using MALDI Biotyper 3.0 software (Bruker Daltonics). This success ratio could be increased up to 91% by lowering the cut-off value of 1.4 for acceptance of identification. However, among the 27 *F. solani* isolates studied, about half of the isolates were not corrected identified to species level (score values <2.0) and one-fourth of the isolates with score values <1.4. The low performance of identification of *F. solani* by MALDI-TOF MS was also noted in our study. Barker et al. (2014) evaluated the performance of MALDI-TOF MS for 77 genetically confirmed isolates of *Paecilomyces* species, the agreement was 92.2% between the molecular and proteomic methods only after supplementation of the MALDI-TOF MS database with type strains. They also indicated that *P. variotii*-like organisms required multilocus DNA interrogations for differentiation and accounted for all of the fungi whose identification was missed by MALDI-TOF MS. In a routine clinical laboratory activity (Cassagne et al., 2011), MALDI-TOF MS-based approach correctly identified 87% (154/177) of the mold isolates analyzed. The failure of identification (12%, 21/177) was found among those not represented in the reference library. In this study, one of the three *R. oryzae* isolates was misidentified as *Mucor circinelloides*.

The performance of identification of many mold genera such as *Aspergillus, Fusarium, Penicillium, Hypocrea/Trichoderma,* and some phytopathogens by MALDI-TOF MS was reviewed comprehensively by Chalupová et al. (2014). However, *P. malneffei* species have not been evaluated in these studies. In this study, using newly created database by MALDI Biotyper for NTUH-3370 strain, 85.7% of the 28 genetically well-documented *P. marneffei* isolates could be identified accurately to species-level. The remaining five isolates were also identified as *P. marneffei* although score values were >1.800 to <2.000.

Summary

Our findings show that new database created for *P. marneffei* MALDI Biotyper could be useful and accurately identify *P. marneffei* isolates, although the number of isolates used in the study was small and only collected mainly from Taiwan. This technique could be incorporated into the work flow of the clinical mycology laboratory for rapid and accurate identification of *P. marneffei*. A continuous updating of current commercial databases provided by MALDI-TOF MS vendors together with the instrumentation as well as building up new databases for specialized research purposes is needed. MALDI-TOF MS is suitable for the routine identification of filamentous fungi in a clinical microbiology laboratory.

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ORIGINAL ARTICLE

Clinical characteristics, microbiology, and outcomes for patients with lung and disseminated nocardiosis in a tertiary hospital

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Background/purpose: *Nocardia* are ubiquitous in the environment worldwide and cause a variety of infections. Clinical manifestations and outcomes of nocardiosis can vary with different populations, host immunity, and presentations. The purpose of this study was to analyze the differences in clinical characteristics, antimicrobial susceptibility, and outcomes for patients with skin, lung, and disseminated nocardiosis.
Methods: We conducted a retrospective survey of culture-proven nocardial infections in 81 patients with invasive nocardiosis over an 18-year period at the National Taiwan University Hospital. The clinical syndromes included skin infections ($n = 44$), localized pulmonary infections ($n = 24$), and disseminated infections ($n = 13$).
Results: Disseminated nocardiosis included lung and brain involvement (7 patients), brain and skin involvement (2 patients), localized brain abscess (1 patient), lung involvement with bacteremia (1 patient), lymphadenitis (1 patient), and liver cirrhosis with spontaneous nocardial peritonitis (1 patient). Eleven (14%) of all patients died due to nocardiosis. In comparison with those with skin infections, patients with lung and disseminated nocardiosis tended to have

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chronic lung disease, malignancy, concomitant bacteremia, were often misdiagnosed as having tuberculosis, were receiving immunosuppressive treatments, and demonstrated an increased mortality. *Nocardia* strains isolated from patients with lung infections or disseminated infections tended to have lower *in vitro* antimicrobial susceptibility than those isolated from skin infections [cefotaxime: 67% (lung) vs. 86% (skin); trimethoprim/sulfamethoxazole: 75% (disseminated) vs. 97% (skin)].

Conclusion: These results highlight the protean disease manifestations and antimicrobial susceptibility of *Nocardia* and indicate the need to address the option of combined antimicrobial therapy for lung and disseminated nocardiosis.

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Introduction

Nocardia is a genus of aerobic, Gram-positive, branching, filamentous, weakly acid-fast bacteria. The organisms contain mycolic acids in the cell wall, are closely related to *Mycobacterium*, and are considered aerobic actinomycetes.[1] *Nocardia* can be found in soil, rotten plants, dust, or water and can infect humans via skin inoculation or respiratory inhalation. There are 88 species of *Nocardia* known at present, 46 of which have been recorded as human pathogens.[1] The most common species are *Nocardia asteroides* and *Nocardia brasiliensis*. *Nocardia* usually infect hosts with impaired cellular immunity.

Nocardiosis is an acute or chronic suppurative disease. The confounding factors associated with nocardiosis include organ transplantation, human immunodeficiency virus (HIV) infection, malignancy, and chronic lung disease.[2–4] Coinfection with *Aspergillus*, cytomegalovirus, or *Mycobacterium* have also been reported.[5–7] Insidious nocardial infections are often misdiagnosed as *Actinomycosis*, *Mycobacterium*, *Cryptococcus*, or *Toxoplasma* infections or as malignancies. Patient outcome is affected by a delay in diagnosis,[8] the presence of concomitant diseases,[5] the particular species of *Nocardia*,[4] and inappropriate therapy.[9]

We reviewed the clinical characteristics, treatment, antimicrobial susceptibility, and outcomes of patients with nocardiosis at a university hospital in northern Taiwan. The objective was to determine the differences in clinical presentation, antimicrobial characteristics, and outcomes among patients with lung and disseminated nocardiosis compared to those with skin infections.

Patients and methods

Study settings

The study was conducted at the National Taiwan University Hospital (NTUH), a major teaching hospital in northern Taiwan with a total capacity of 2500 beds. This hospital offers primary and tertiary care for patients with acute diseases. All culture-proven cases of nocardiosis diagnosed at NTUH from January 1988 through December 2006 were included and in-hospital outcomes as the end point by chart review.

Definition of nocardiosis

This was a retrospective analysis. According to chart records, the *Nocardia* isolates were obtained from various clinical specimens, including pus from skin abscesses, sputum, lung aspirate, pleural effusion, resected pleural tumor, brain abscess, blood, and ascites. Nocardiosis was considered a definite diagnosis when *Nocardia* was repeatedly isolated or one isolate from sterile site. The participants of this study were divided on the basis of infection type into cutaneous, pulmonary only, and disseminated cases. Histological culture of the bacteria on bronchoalveolar lavage or lung aspiration was required for diagnosis of pulmonary nocardiosis. Findings of infection in the brain, two or more noncontiguous organs, or in the blood were required for diagnosis of disseminated nocardiosis.[10] Nocardiosis was defined as a cause of death if the clinical condition deteriorated and the patients died.

Microbiological methods

Specimens of suspected *Nocardia* species were smeared onto slides, Gram-stained to detect the presence of predominant Gram-positive filamentous organisms, acid-fast stained using a modified method, and incubated for at least 2 weeks on agar plates. Blood was cultured using the BACTEC blood culture system (Becton, Dickinson, and Company, Sparks, MD, USA). Identification of the isolates to the species level was performed using conventional biochemical tests for *Nocardia*, which involved hydrolysis of casein, xanthine, hypoxanthine, and tyrosine.[11] Antimicrobial susceptibility testing was performed using the disk diffusion method with amikacin, minocycline, cefotaxime, trimethoprim–sulfamethoxazole (TMP/SMX), ampicillin, gentamicin, or ciprofloxacin on Mueller–Hinton agar according to the National Committee for Clinical Laboratory Standards criteria.[12] From 2008, identification to the species level were confirmed by 16S rRNA gene sequence analysis and susceptibility testing was performed using the E-test method at NTUH.

Data collection and other definitions

This was a cohort review that involved analysis of relevant clinical, demographic, chest radiographic, and laboratory data. Data on the presence of underlying diseases, types of

infection, immunosuppressive therapy, source of *Nocardia* isolation, antimicrobial therapy, surgical intervention, and final outcome were recorded. Patients were considered to have concomitant bacteremia if non-nocardial species were present in blood cultures and the patient had symptoms compatible with bacteremia. Immunosuppressive therapy was defined as antineoplastic therapy, treatment with other immunosuppressants (such as azathioprine, cyclosporin, mycophenolate mofetil, tacrolimus, or sirolimus) within 6 weeks prior to a positive culture for *Nocardia*, or corticosteroid therapy. A dose of 20 mg or more of prednisolone daily for at least 2 weeks or 30 mg of prednisolone for at least 1 week prior to a positive culture for *Nocardia* was the definition of corticosteroid therapy. Sepsis was defined according to the definitions of the American College of Chest Physicians/Society of Critical Care Medicine.[13]

Efficacy of antibiotic treatment was assessed on the basis of improvement in clinical signs and symptoms and on *in vitro* sensitivity to antibiotics. Antibiotic regimens were also divided into single agent therapies (TMP/SMX, penicillin/cephalosporin, or minocycline) and combined therapies (TMP/SMX, carbapenem, or minocycline in combination with other antibiotics).

Statistical analysis

The sample size of patients with disseminated nocardiosis was only 13 in this research, and only three out of 13 disseminated cases showed no lung involved. Because pulmonary infection and disseminated infection are hard to differentiate and share more common clinical and microbiology features, patients with lung and disseminated nocardiosis were pooled in the same category to compare clinical characteristics, outcomes, and antimicrobial results of the isolates with patients with skin infections. Microsoft Excel was utilized for all statistical analyses. The proportions were compared using Chi-square test. A p value <0.05 was considered significant.

Results

According to the laboratory reports and chart recordings, *Nocardia* was present in 87 patients, but six of these patients were defined as colonized based only on the report of sputum analysis without invasive procedures or new chest radiological lesions. All six patients had chronic lung diseases and received first generation cephalosporin and corticosteroids. The remaining 81 patients with culture-proven invasive nocardiosis were diagnosed at NTUH during the study period. Clinical manifestations of these 81 patients included skin involvement only ($n = 44$), pulmonary nocardiosis without dissemination ($n = 24$), and disseminated with or without brain abscess ($n = 13$).

A total of 64% of patients (28/44) with skin nocardiosis had nodular lymphangitis, 30% (13/44) had folliculitis or carbuncles, 5% (2/44) had pyoderma, and 2% (1/44) had mycetoma. Half of the patients with cutaneous infections (22/44) had lesions on their lower extremities, 36% (16/44) had infection of an upper extremity, and 14% (6/44) patients had facial infections. Except trauma (2/44), other predisposing factors for skin nocardiosis were insect bite (3/44), dog bite (1/44), burns (2/44), and tinea pedis (2/44) with secondary nocardial wound infection according to chart recording.

A total of 77% of patients (10/13) with multiple organ involvement had brain abscesses. Of these, seven had both brain abscess and lung involvement, two had brain abscess and skin involvement, and one had a localized brain abscess only. Of the three other multi-system cases, each presented with lung involvement with nocardial bacteremia, lymphadenitis, or liver cirrhosis with spontaneous nocardial peritonitis, independently. Neurological deficits prompted surgery in 60% of the patients (6/10) with brain abscesses. Of these six patients, three presented with thalamic, two with parietal, and one with frontal lobe abscesses. Brain magnetic resonance imaging (MRI) indicated multiple brain microabscesses in another four patients, even though these patients had no neurological signs.

Table 1 shows the basic demographics, clinical features, and microbiological data for the patients stratified by infection type. The male-to-female ratio was 1.4:1 and the mean age was 54.6 years (range, 3—89 years). The most common underlying disease was chronic lung disease (14%, 11/81); others included diabetes mellitus, malignancy, autoimmune diseases, transplantation recipients, and acquired immunodeficiency syndrome (AIDS). Chronic lung disease included bronchiectasis (6/81), bronchial asthma (4/81), and chronic obstructive pulmonary disease (1/81). Malignancy included chronic myelocytic leukemia (2/81) and one each with breast carcinoma, cervical carcinoma, rectal carcinoma, gingival carcinoma, gastric carcinoma, or hepatic cell carcinoma. Autoimmune disease included systemic lupus erythematosus (4/81), polymyositis (1/81), and Paget's disease (1/81). Transplantation recipients included renal (3/81), heart (1/81), and bone marrow transplantation (1/81). A total of 40% of patients (32/81) received immunosuppressive therapy, with 30% (24/81) having received corticosteroid therapy.

Nocardia were identified to species level using conventional methods and included 46 *N. brasiliensis* (57%), 32 *N. asteroides* (40%), one *Nocardia otitidiscaviarum* and two unidentified species. Cutaneous nocardiosis occurred in 54% of cases (44/81), 91% (40/44) of which were caused by *N. brasiliensis*. Only 9% of cutaneous cases (4/44) were caused by *N. asteroides*, the species most commonly found in pulmonary and disseminated nocardiosis. Localized pulmonary nocardiosis (24/81) was associated with the highest incidence (63%, 15/24) of concomitant bacteremia or receiving immunosuppressive treatment (63%, 15/24) and the highest incidence of steroid use. Among the pulmonary group, 54% (13/24) had sepsis. A total of 79% of patients (19/24) were infected by *N. asteroides*, 13% (3/24) by *N. brasiliensis*, 4% (1/24) by *N. otitidiscaviarum*, and 4% (1/24) by an unidentified species. *Mycobacterium tuberculosis* and *Nocardia* were recovered together from lung tissues or sputum in 21% of patients (5/24) in the pulmonary group. The organisms causing concomitant bacteremia were *Staphylococcus aureus*, *Salmonella* O4 (GrB), *Chryseobacterium meningosepticum*, *Haemophilus parainfluenzae*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Candida albicans*, and *Penicillium marneffei*. One third of the patients (8/24) in the pulmonary group died.

Table 1 Demographic and clinical characteristics of patients with skin, lung, and disseminated nocardiosis.[§]

Variable	Total (n = 81)	Skin (n = 44)	Lung (n = 24)	Disseminated (n = 13)	Lung + disseminated (n = 37)	p
Age (y, mean ± SD)	54.6 ± 20.0	54.5 ± 21.8	53.4 ± 18.7	57.4 ± 17.0	54.8 ± 18.0	
Males	47 (58)	24 (55)	16 (67)	7 (54)	23 (62)	0.49
Species of *Nocardia*						
asteroides	32 (40)	4 (9)	19 (79)	9 (69)	28 (76)	
brasiliensis	46 (57)	40 (91)	3 (13)	3 (23)	6 (16)	<0.01*
otitidiscaviarum	1 (1)	0 (0)	1 (4)	0 (0)	1 (1)	
unidentified	2 (2)	0 (0)	1 (4)	1 (8)	2 (5)	
Underlying disease						
Chronic lung disease	11 (14)	1[a] (2)	9[b] (38)	1[c] (8)	10 (27)	<0.05*
Diabetes mellitus	8 (10)	6 (14)	1 (4)	1 (8)	2 (5)	0.22
Malignancy	8 (10)	1[d] (2)	5[e] (21)	2[f] (15)	7 (19)	<0.05*
Autoimmune disease	6 (7)	3[g] (7)	2[h] (8)	1[i] (8)	3 (8)	0.83
Transplantation	5 (6)	1[j] (2)	2[k] (8)	2[l] (15)	4 (11)	0.11
AIDS	5 (6)	1 (2)	2 (8)	2 (15)	4 (11)	0.11
Immunosuppressive therapy	32 (40)	11 (25)	15 (63)	6 (46)	21 (57)	<0.05*
Corticosteroid usage	24 (30)	8 (18)	11 (46)	5 (38)	16 (43)	<0.05*
Concomitant bacteremia	18 (22)	2 (5)	15 (63)	1 (8)	16 (43)	<0.05*
Coinfection with TB	8 (9)	2 (5)	5 (21)	1 (8)	6 (16)	0.11
Misdiagnosis as TB	13 (16)	1 (2)	9 (38)	3 (23)	12 (32)	<0.05*
Sepsis	16 (36)	1 (2)	13 (54)	2 (15)	15 (41)	<0.05*
Antibiotics treatment						
Single drug therapy	47 (58)	32 (73)	11 (46)	4 (31)	15 (41)	<0.05*
TMP-SMX	33 (41)	21 (48)	9 (38)	3 (23)	12 (32)	0.16
Penicillin or cephalosporin	12 (15)	9 (20)	2 (8)	1 (8)	3 (8)	0.12
Minocycline	2 (2)	2 (5)	0 (0)	0 (0)	0 (0)	0.19
Combined therapy	33 (41)	11 (25)	13 (54)	9 (69)	22 (59)	<0.05*
TMP-SMX + other[m]	30 (37)	11 (25)	11 (46)	8 (61)	19 (51)	<0.05*
Carbapenem + other[n]	2 (2)	0 (0)	2 (8)	0 (0)	2 (5)	0.12
Minocycline + cephalosporin	1 (1)	0 (0)	0 (0)	1 (8)	0 (0)	0.27
No therapy	1 (1)	1 (2)	0 (0)	0 (0)	0 (0)	0.36
Treatment inadequacy	13 (16)	7 (16)	4 (17)	2 (15)	6 (16)	0.97
Duration of therapy (d)						
Mean ± SD	78.9 ± 88.8	59.5 ± 51.6	62.5 ± 54.7	175.8 ± 158.6	102.3 ± 115.3	
Median	62	56	51	180	76**	
Range	3–547	3–195	12–226	7–547	7–547**	
Operation	25 (31)	16 (36)	3 (13)	6 (46)	9 (24)	0.24
Overall mortality due to nocardiasis	11 (14)	0 (0)	8 (33)	3 (23)	11 (30)	<0.05*
60 days after diagnosis	9 (11)	0 (0)	7 (29)	2 (15)	9 (24)	<0.05*

§Comparison of lung and disseminated group with skin group by Chi-square test.
*Statistically significant.
**Because of the large standard deviation and lack of normal distribution, median and range are a better way to show the significance.
AIDS = acquired immunodeficiency syndrome; CA = carcinoma; COPD = chronic obstructive pulmonary disease; SD = standard deviation; SLE = systemic lupus erythematosus; TB = *Mycobacterium tuberculosis*; TMP-SMX = trimethoprim/sulfamethoxazole.

[a] Bronchiectasis.
[b] Asthma (4 patients), bronchiectasis (5 patients).
[c] COPD.
[d] SLE (2 patients), polymyositis (1 patient).
[e] SLE, Paget's disease.
[f] SLE.
[g] Breast CA.
[h] Cervical CA, rectal CA, gingival CA, chronic myelocytic leukemia (2 patients).
[i] Gastric CA, hepatic cell CA.
[j] Renal.
[k] Heart, bone marrow.
[l] Renal.
[m] Other = amikacin; penicillin; fluoroquinolone; erythromycin; amikacin + carbapenem, minocycline, or penicillin; minocycline + 3rd generation cephalosporin; or amikacin + carbapenem + 3rd generation cephalosporin.
[n] Penicillin or vancomycin.

In contrast to the pulmonary group, only 8% of patients (1/13) in the disseminated group had polymicrobial infection. In the disseminated group, 23% (3/13) of the patients died.

The most common treatment agent, TMP/SMX, was used as a single (41%, 33/81) agent or in combination therapy (37%, 30/81). Most cutaneous cases (73%, 32/44) were treated with a single agent. Combination therapy was more commonly used in the disseminated cases (69%, 9/13). For the pulmonary cases, single (46%, 11/24) and combination therapy (54%, 13/24) were nearly equally common.

In comparison with the skin infection group, patients with lung and disseminated nocardiosis tended to have *N. asteroides* infections and an underlying disease, including chronic lung disease or malignancy. These patients also presented with clinically critical conditions, such as sepsis, and were often initially misdiagnosed as having tuberculosis. The patients also tended to receive immunosuppressive treatment and combined antimicrobial therapy, and they also had increased mortality.

In total, 14% (11/81) of the patients died from nocardiosis. The mean survival of the patients who died from nocardiosis was 42 days (range, 1—161 days), with 55% (6/11) of these patients having died within 25 days and 82% (9/11) within 60 days after diagnosis.

Table 2 shows that *Nocardia* were isolated and cultured from various sources. The diagnosis of lung nocardiosis depended on culture of lung aspiration (63%, 15/24), sputum (38%, 9/24), pleural effusion (21%, 5/24), bronchoalveolar lavage (13%, 3/24), postpneumonectomy bronchial tissue (8%, 2/24), or pleural mass (4%, 1/24).

Table 3 shows the radiographic results of the 34 patients with lung involved nocardiosis (including disseminated type with lung involvement). Consolidation (71%, 24/34) was the most common presentation of the radiographic findings, followed by cavitation (53%, 18/34), nodules or masses (41%, 14/34), pleural effusion (38%, 13/34), infiltration (9%, 3/34), extension to chest wall (3%, 1/34), and pleura mass (3%, 1/34). In total, 53% (18/34) of these patients had bilateral lung involvement.

Table 4 shows the antimicrobial susceptibility of the *Nocardia* species from the different infectious sites. The isolates showed susceptibility to amikacin (97%), TMP/SMX (94%), gentamicin (80%), minocycline (79%), and cefotaxime (79%), but poor susceptibility to ampicillin (17%) and ciprofloxacin (12%). All of the isolates were resistant to erythromycin. The *Nocardia* strains isolated from patients with disseminated infections (75%, 6/8) tended to have lower *in vitro* antimicrobial susceptibility to TMP/SMZ than those from skin (97%, 34/35) or lung infections (95%, 18/19). It is worth noting that the isolates from lung infections had lower susceptibility to cefotaxime (67%, 10/15) than those from skin infections (86%, 30/35). Based on these analyses, the isolates from lung and disseminated infections showed significantly lower susceptibility (59%, 16/27) to gentamicin than those isolated from skin infections (97%, 34/35).

Discussion

Our results show that the predisposing factors for nocardial infection include chronic lung disease, diabetes mellitus, malignancy, autoimmune disease, HIV infection, and transplantation recipients. Although there have been several published papers describing clinical and microbiologic features of nocardiosis in Taiwan, disseminated nocardiosis is rarely reported.[14—16] In this report, we highlighted the propensity of central nervous system (CNS) involvement [77% (10/13)] in disseminated nocardiosis. In comparison with those with skin infections, patients with lung and disseminated nocardiosis tended to have chronic lung disease, malignancy, concomitant bacteremia, were often misdiagnosed as having tuberculosis, were receiving immunosuppressive treatments, and demonstrated an increased mortality. We also note that *Nocardia* strains isolated from patients with lung infections or disseminated infections tend to have lower *in vitro* antimicrobial susceptibility than those isolated from skin infections.

Our study population differed from those in previous studies in that slightly more than half of our patients had cutaneous nocardiosis and more than 90% of these cutaneous cases were caused by *N. brasiliensis*. Among our patients, the most common cutaneous presentation was nodular lymphangitis, which manifested as nodular lesions that progressed in a sporotrichoid pattern as cellulitis, ulcerative or bullous lesions, granuloma, and abscesses. In Western countries, *N. asteroids* accounts for 60—80% of all cases and *N. brasiliensis* is seldom seen. In fact, *N. brasiliensis* is rarely found in temperate climates, but it has been recovered from the soil in many tropical and subtropical countries such as Taiwan.[17—19] *N. brasiliensis* has been reported to be mostly associated with cutaneous disease. The patient with mycetoma on the lower extremity in our cohort had chronic ulcers for over 10 years. Due to sulfa granules on pathological findings, the physician had misdiagnosed the lesions as an *Actinomycetes* infection. Subsequent identification of *Nocardia* allowed this patient to receive the appropriate management. For proper diagnosis, culture analysis is necessary. In total, 45% of the cases of skin nocardiosis in this cohort had no underlying disease. All of the patients with skin nocardiosis had survived.

Most of the remainder of our patient population had pulmonary nocardiosis, either alone (24 cases) or as part of

Table 2 Diagnostic methods of cultured-proven nocardiosis (81 patients).

	No. of patients
Skin pus	46
Lung aspiration	15
Sputum	9
Brain abscess	6[a]
Pleural effusion	5
Bronchial alveolar lavage	3
Surgical lung tissue	2
Pleural mass	1
Lymph node	1
Blood	1
Ascites	1

[a] Proven by surgical specimens in six patients; another four patients showed multiple microabscess from brain magnetic resonance imaging.

Table 3 Chest radiographic findings of 34 patients with lung-involved nocardiosis (both localized lung and disseminated nocardiosis).

	No. of patients (%)
Consolidation	24 (71)
Cavitation	18 (53)
Nodules or masses	14 (41)
Pleural effusion	13 (38)
Infiltration	3 (9)
Extension to chest wall	1 (3)
Pleural mass	1 (3)
Bilateral lung involvement	18 (53)

disseminated disease (10 patients). Only three disseminated cases showed no lung involvement. Chronic lung disease (38%) was the most common underlying disease among those with isolated pulmonary disease. We cannot meaningfully compare our results with those of previous studies because the prevalence of underlying diseases in a study sample depends greatly on their prevalence in the populations from which the sample was drawn. For example, in a report from a large county hospital in Miami, FL, USA, where there is a high incidence of AIDS, 76% of the patients with nocardiosis (average age 40 years) had AIDS as an underlying disease.[20] By contrast, in a population (average age 65 years) from two tertiary hospitals in Australia, lung disease was the most common underlying disease (63%) and only 3% of patients had AIDS.[21] In a completely different patient population from the Central Anatolia region of Turkey, the predominant (44%) underlying medical condition in nocardiosis cases (average age of 31 years) was renal transplantation.[22]

Inhalation is thought to be responsible for most cases of invasive pulmonary nocardiosis. The prevalence of respiratory tract colonization may be much higher in patients with underlying chronic lung disease, such as chronic bronchitis, emphysema, asthma, or bronchiectasis, and colonization may be the reason for development of disease.[4] The chest X-rays of patients with lung nocardiosis had nonspecific findings of consolidation with or without cavitation, masses or nodules, empyema, or pleural effusion. Patients who are also immunocompromised, invasive diagnostic procedures such as bronchoalveolar lavage, lung tissue aspiration, or surgical dissection are essential for early diagnosis, especially in regards to disseminated nocardiasis.[23]

In previous reports, pulmonary tuberculosis coexisting with nocardiosis is rare, although cases have been reported in India.[24] Five of our pulmonary patients had active pulmonary tuberculosis in addition to nocardial infection, presumably because Taiwan has an estimated incidence rate of pulmonary tuberculosis infection (54.5 new reported tuberculosis/100,000 in 2011).[25] Concomitant infection with *Mycobacterium* species has been implicated as a risk factor for development of nocardiosis in HIV-infected patients.[26] Increased reporting of nocardiosis in Taiwan will be required to determine whether a similar association exists between nocardial disease and infection with *M. tuberculosis*.[14]

Pulmonary disease and diseases involving depressed immune function (63%) are risk factors for the development of nocardiosis. Conditions reported to be predictive of increased mortality from nocardiosis are CNS disease,[8,27] severe immunodeficiency,[22] and steroid therapy.[22,28] In our patient population, death rates were higher in the pulmonary disease group (33%) than in the disseminated disease group (23%), and 77% of the patients had CNS involvement. Most deaths (82%) occurred during the first 2 months after diagnosis and even they received TMP/SMX (8 patients) or carbapenem (5 patients) based combination of antimicrobial therapy. Virtually all patients in these groups had an underlying disease (81%) that presumably increased the risk of developing nocardiosis. Sepsis was either a direct cause of death in patients with nocardiosis. Immunosuppressive therapy increases the risk of concomitant bacteremia by other microorganisms [*S. aureus*, *Salmonella* O4 (GrB), *C. meningosepticum*, *H. parainfluenzae*, *S. pneumoniae*, *K.*

Table 4 Comparison of the antimicrobial susceptibility of *Nocardia* species isolated from patients with different infection sites.[a]

Antimicrobial agents		Susceptible no. of cases/test isolates (n^b percent sensitive)				
	Total susceptibility (%)	Skin ($n = 44$)	Lung ($n = 24$)	Disseminated ($n = 13$)	Lung + disseminated ($n = 37$)	p
Amikacin	97	36/36 (100)	16/17 (94)	6/7 (86)	22/24 (92)	0.08
TMP/SMZ	94	34/35 (97)	18/19 (95)	6/8 (75)	24/27 (89)	0.19
Minocycline	79	30/37 (81)	14/18 (78)	5/7 (71)	19/25 (76)	0.63
Cefotaxime	79	30/35 (86)	10/15 (67)	6/8 (75)	16/23 (70)	0.14
Gentamicin	80	34/35 (97)	11/19 (58)	5/8 (63)	16/27 (59)	<0.01*
Ampicillin	17	6/36 (17)	3/19 (16)	2/9 (22)	5/28 (18)	0.90
Ciprofloxacin	12	3/36 (8)	3/15 (20)	1/9 (11)	4/24 (17)	0.32
Erythromycin	0	0/36 (0)	0/18 (0)	0/6 (0)	0/54 (0)	—

*Statistical significance.
TMP-SMX = trimethoprim/sulfamethoxazole.
[a] Comparison by Chi-square test.
[b] Percentages indicate the percent of cases in which the isolate was sensitive to the antibiotic tested; numbers represent the total number of cases in which the isolate was tested against the specific antibiotic.

pneumoniae, *P. mirabilis*, *C. albicans*, and *P. marneffei*], which in turn can lead to death or increase the likelihood of death from nocardiosis.

There is little information on the effect of poor immune function on the development of nocardiosis. Many patients with nocardiosis (transplant recipients and HIV positive patients) are given an anti-nocardial drug (TMP/SMX) for prophylaxis or treatment of *Pneumocystis jiroveci* infections commonly present in these populations. TMP/SMX resistance in patients positive for HIV in this cohort was 25%. The patients positive for HIV that have received TMP/SMX prophylaxis and are concurrently infected with *Nocardia* species will be of substantial interest in future studies.

In this study, 77% of the patients, were treated with TMP/SMX, but in some regions, more than half of nocardial strains are resistant to this drug.[29] Although *in vitro* tests showed that 93% of our isolates were sensitive to this drug and that 97% were sensitive to amikacin, the demonstration of *in vitro* susceptibility is not always equivalent to clinical effectiveness.[4] In this cohort, *Nocardia* strains isolated from patients with lung or disseminated infections tended to be resistant. TMP/SMX combined with imipenem, or third generation cephalosporins, or minocycline with or without amikacin have been the primary regimens for the treatment of pulmonary, disseminated, or brain nocardiosis or in patients receiving immunosuppressive agents.[3] The mortality of patients with lung nocardiosis receiving single or combined therapy in this cohort was 33%. The need for combination therapy in patients with lung or disseminated nocardiosis for empirical therapy prior to antimicrobial susceptibility has been suggested.

Due to *Nocardia* tropism towards cerebral tissues, if a physician is faced with lung or disseminated nocardiosis, brain MRI should be arranged to rule out brain abscess.[1] In this cohort, four of 10 patients with brain nocardiosis revealed microabscesses in MRI findings, and these patients demonstrated no neurological deficit.

The main limitation of this cohort study is its retrospective nature. The antimicrobial susceptibility data for each isolate are not complete and especially the sensitivity data for carbapenem group, which may be crucial for treatment of severe infection and central nervous system infection. Molecular techniques have been used to identify the species and predict the antimicrobial susceptibility patterns in previous studies, but molecular typing was not available for routine identification in every hospital.[18]

The incidence rate of nocardiosis is considered to be increasing, due to immunosuppressing agents prescribed to patients with chronic lung disease, malignancy, or autoimmune disease as well as transplantation recipients.[14] The diagnosis depends on a high degree of suspicion. Smears and culture remain the principal method for diagnosis. If there is a clinical suspicion of *Nocardia* infection, physicians should inform the laboratory of the possibility of *Nocardia* species, as the laboratory must prolong culture duration to 3 weeks because some *Nocardia* species are slow growing.[1]

Lung and disseminated nocardiosis can be a significant cause of morbidity and mortality, especially in patients with underlying diseases, including chronic lung disease and malignancy, and patients receiving immunosuppressive agents. Early recognition of nocardiosis enables a more rapid initiation of appropriate antimicrobial therapy and a more favorable outcome. Our results indicate that isolates from patients with lung or disseminated nocardiosis tend to be resistant to antimicrobials, which is important when considering the option of treatment with combined antimicrobial agents.

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RESEARCH ARTICLE

A Clinical Algorithm to Identify HIV Patients at High Risk for Incident Active Tuberculosis: A Prospective 5-Year Cohort Study

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Abstract

Background

Predicting the risk of tuberculosis (TB) in people living with HIV (PLHIV) using a single test is currently not possible. We aimed to develop and validate a clinical algorithm, using baseline CD4 cell counts, HIV viral load (pVL), and interferon-gamma release assay (IGRA), to identify PLHIV who are at high risk for incident active TB in low-to-moderate TB burden settings where highly active antiretroviral therapy (HAART) is routinely provided.

Materials and Methods

A prospective, 5-year, cohort study of adult PLHIV was conducted from 2006 to 2012 in two hospitals in Taiwan. HAART was initiated based on contemporary guidelines (CD4 count < = 350/μL). Cox regression was used to identify the predictors of active TB and to construct the algorithm. The validation cohorts included 1455 HIV-infected individuals from previous published studies. Area under the receiver operating characteristic (ROC) curve was calculated.

Results

Seventeen of 772 participants developed active TB during a median follow-up period of 5.21 years. Baseline CD4 < 350/μL or pVL ≥ 100,000/mL was a predictor of active TB

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(adjusted HR 4.87, 95% CI 1.49–15.90, $P = 0.009$). A positive baseline IGRA predicted TB in patients with baseline CD4 $\geq$ 350/μL and pVL < 100,000/mL (adjusted HR 6.09, 95% CI 1.52–24.40, $P = 0.01$). Compared with an IGRA-alone strategy, the algorithm improved the sensitivity from 37.5% to 76.5%, the negative predictive value from 98.5% to 99.2%. Compared with an untargeted strategy, the algorithm spared 468 (60.6%) from unnecessary TB preventive treatment. Area under the ROC curve was 0.692 (95% CI: 0.587–0.798) for the study cohort and 0.792 (95% CI: 0.776–0.808) and 0.766 in the 2 validation cohorts.

Conclusions

A validated algorithm incorporating the baseline CD4 cell count, HIV viral load, and IGRA status can be used to guide targeted TB preventive treatment in PLHIV in low-to-moderate TB burden settings where HAART is routinely provided to all PLHIV. The implementation of this algorithm will avoid unnecessary exposure of low-risk patients to drug toxicity and simultaneously, reduce the burden of universal treatment on the healthcare system.

Introduction

Tuberculosis (TB) remains the major cause of death in people living with HIV (PLHIV) worldwide [1]. The World Health Organization (WHO) estimates that 13% of the 8.6 million new TB cases in 2012 was co-infected with HIV, causing almost a quarter of the 1.3 million TB-related deaths [2]. HIV infection accelerates progression of latent TB infection into active TB disease [3]. HIV-infected persons have an approximately 30 times increase in the incidence of active TB [4]. While the use of highly active antiretroviral therapy (ART) reduces the risk of TB by 70%–90%, the incidence of TB remains two to four-fold higher than HIV-negative populations [5–9]. An important strategy to further reduce HIV-associated TB is to provide isoniazid preventive therapy (IPT) [8, 10], which has been demonstrated to decrease TB incidence by 27%–37% in patients receiving ART, regardless of the tuberculin skin test (TST) or interferon gamma releasing assay (IGRA) results [11–13].

WHO currently recommends that all PLHIV without evidence of active TB in resource-constrained, high-TB-incidence settings, should be provided with IPT [14]. It remains uncertain, however, whether such recommendations should be extended to HIV-infected positive persons who are not known TB contacts in low-to-moderate TB-burden settings where ART is routinely used to treat all PLHIV. In such settings, untargeted IPT will place large numbers of persons, who may never benefit from IPT, at risk for adverse drug reactions [15]. The preventive treatment would be justifiable only if IPT was targeted to those with significantly increased risk of TB. Nevertheless, no single test available currently can accurately predict the risk of TB in PLHIV.

In HIV patients under profound immunosuppression status, both TST and IGRA may yield false-negative or indeterminate results [3, 16–17]. Studies showed that TST failed to identify 61% (20/33)[12] to 66% (27/41)[13] of PLHIV who develop incident active TB within 4 years of testing and thus may benefit from IPT, and IGRA failed to identify 53% (21/40)[13] of such patients. We hypothesized that the accuracy of predicting risk of TB can be improved by incorporating the patients' CD4 cell count and HIV viral load as predictors, since both are known markers for HIV-associated immunosuppression that increase the risk of incident active TB; while for those with relatively intact immunity, tests for latent TB infection should still be

useful to predict risk of active TB. Whether this algorithm approach can be used to guide targeted IPT for PLHIV has not been previously investigated.

Taiwan is a moderate-TB-burden country (annual TB incidence: 53/100,000 population in 2012)[18], where ART is routinely used to treat all eligible PLHIV since 1997. Taiwan CDC has a policy endorsing the use of IPT in PLHIV, but similar to many countries worldwide, uptake has been limited due to lack of programs to implement the policy [19]. Major concerns against the use of IPT include unnecessary isoniazid hepatotoxicity (8%) [20] when the risk of TB has been greatly reduced by both ART [5] and routine childhood BCG vaccination [21–22], as well as the lack of durable protection of IPT in PLHIV [12–13]. These concerns led to an extremely low uptake of latent TB infection testing and treatment among PLHIV who are not known TB contacts in Taiwan.

This prospective, cohort study aimed to develop and validate a simple, easy-to-use clinical algorithm, based on baseline CD4 cell count, plasma HIV viral load, and IGRA results, to identify those HIV patients who are at an increased risk of active TB and thus are most likely to benefit from IPT in an intermediate TB burden country.

Methods

Ethical Statement

Institutional review boards in both hospitals (Institutional Review Board, Kaohsiung Veterans General Hospital and Institutional Review Board, E-Da Hospital) approved the study protocol. All of the participants gave written informed consent.

Settings

This study was conducted at a medical center, Kaohsiung Veterans General Hospital (Kaohsiung, Taiwan), and a regional hospital, E-Da Hospital (Kaohsiung, Taiwan) in southern Taiwan.

Study Design

This prospective, cohort study invited all HIV-infected adult patients who attended outpatient clinics at the study hospitals, from Jan, 2006 to Dec, 2010. The primary outcome was incident active TB. None of the participants were involved in TB contact investigations at the time of enrollment. Validation of the algorithm was done using 2 separate, HIV-infected cohorts in Northern Taiwan from previous published studies [23–24].

Baseline Evaluations

At study entry, clinical evaluation, chest radiograph, and IGRA were performed. Information on demographic data, HIV risk factors, history of BCG vaccination, previous exposure to TB, and past TB disease were obtained by using a standard questionnaire. Previous exposure to TB was considered present if the patient reported ever living with or having contact with a person with active TB in their lifetime. Those with active TB at baseline, as evident by compatible clinical symptoms (such as fever, chills, productive cough, anorexia, body weight loss, etc.), radiologic findings and mycobacterial cultures, were given treatment and not eligible for the study. Those who developed TB within the 60 days run-in period after study entry were assumed to have active TB at baseline and were excluded. Baseline CD4 cell counts and HIV viral loads (tested within three months before study entry), and whether the patients were already receiving ART, were obtained from the medical records. All participants with a CD4 cell count < 350 cells/μL were treated with ART according to contemporary national guidelines

[25]. The IGRA results were provided to the physician in charge, and patients testing positive for IGRA were offered the choice to receive IPT or not, after discussion with their primary care physicians. IPT was initiated at the discretion of the in-charge physician.

IGRA

QuantiFERON-TB GOLD test (Cellestis Ltd, Melbourne, Australia) was performed and interpreted according to the manufacturer's instructions. The result was positive if the IFN-γ response to TB antigen minus background levels was $\geq$ 0.35 IU/mL, negative when the IFN-γ response to TB antigen minus background levels was $<$ 0.35 IU/mL, and indeterminate if the background IFN-γ level was $\geq$ 8.0 IU/mL, or IFN-γ response to phytohaemagglutinin $<$ 0.5 IU/mL.

Follow Up and Ascertainment of Outcome

All patients were scheduled to be followed up every three months, until they developed active TB disease (event), died from other causes (censored), started IPT (censored) or were censored on Dec 31, 2012. The majority (98.3%) was followed up for a minimum of two years. Because participants may seek medical treatment for active TB in other hospitals, we used the national TB registry to ascertain the outcome during the study period.

Validation Cohort

Validation of the clinical algorithm was done using 2 separate, HIV-infected cohorts, derived from previously published studies [23–24]. Validation cohort 1 consisted of 912 HIV-infected persons from 3 HIV outpatient clinics and 2 prisons located in Northern Taiwan [23], and validation cohort 2 consisted of 543 HIV-infected patients at the HIV clinic in another medical center in Northern Taiwan, for whom CD4 cell counts, HIV viral load and IGRA results are available [24]. Both cohorts did not receive IPT, and used T-SPOT.TB test (Oxford Immunotec, Oxford, United Kingdom) as the interferon-gamma release assay. T-SPOT.TB also uses ESAT-6 and CFP-10 as the specific antigens for *M.tuberculosis* for stimulation of lymphocytes, and uses ELISPOT for enumerating lymphocytes with interferon gamma release. Area under the receiver operating characteristic (ROC) curve was calculated.

Statistical Analysis

All statistical analyses were conducted using Stata release 10 (STATA Corp, College Station, TX, USA). A *P* value of less than 0.05 was considered significant, and *P* values were two-sided. CD4 cell counts cut-off of 350 cells/μL and an HIV viral load cut-off of 100,000 copies/mL was chosen based on universally accepted cut-offs; CD4 $<$ 350 cells/μL was the cut-off to initiate antiretroviral therapy, and an HIV viral of $>$ 100,000 copies/mL represented a high viral load that may affect antiretroviral treatment response. We used Cox proportional hazards regression model to analyze the predictors for incident active TB. In multivariable models, age and baseline CD4 count was adjusted for, since CD4 count is a known risk factor for IGRA positivity and reactivation of TB. The forward stepwise selection method and likelihood ratio test was used to select the final model. Validation was done by comparing the area under the ROC curve of the 2 separate, HIV-infected cohorts.

Results

Participants

A total of 774 adult HIV patients agreed to be enrolled. Two were excluded from study (one had active TB disease at entry and the other developed TB within 42 days) (Fig 1). The mean

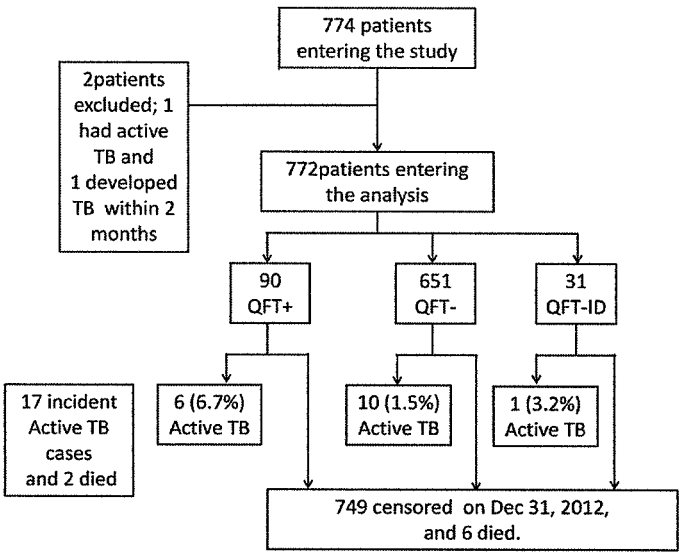


Fig 1. Flowchart of HIV-infected patients who entered the study, and the proportion who developed tuberculosis, stratified by results of interferon-gamma release assay (IGRA).

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age of the 772 participants was 36.8 years (SD 9.0, range 20.0–77.2), most were men (744, 96.4%). HIV risk factors included injection drug users (IDUs) (517, 67.0%), unprotected sexual transmission (men who have sex with men in 187, 24.2% and heterosexuals in 62, 8.0%) and others (6, 0.8%). Nearly all participants had received BCG vaccination (755, 97.8%) (Table 1).

Baseline CD4 Cell Counts and HIV Viral Load

At study entry, 70.6% (545/772) participants had a CD4 cell count of $\geq$ 350 cells/μL. Almost a third (254, 32.9%) in overall was receiving ART, and 41% (93/227) with CD4 cell count < 350 cells/μL was on ART. Many patients were newly diagnosed, and were placed on ART thereafter, reaching 68% (154/227) ART coverage for those eligible for ART based on treatment guidelines at the time (i.e. initiation of ART when CD4 cell count < 350 cells/μL).

The median CD4 cell count was 460 cells/μL (IQR 329–634) and the median $\log_{10}$ plasma HIV viral load was 3.40 copies/mL (IQR 1.74–3.97) (Table 1). Fourteen patients had HIV viral loads > 100,000 copies/mL, of which ten were newly diagnosed with HIV infection, and four had virological failure.

Baseline IGRA Results

IGRA was positive in 90 (11.7%) and indeterminate in 31 (4.0%) of subjects. The median CD4 cell count was significantly higher in patients with positive compared with negative IGRA results (511 vs 459 cells/μL, p = 0.01), and significantly lower in those with indeterminate results (339 vs 459 cells/μL, p = 0.01). Patients with low CD4 cell counts of less than 200 cells/μL were less likely to be IGRA positive (5/66, 7.6% vs 85/706, 12.0%, p = 0.02), and had more indeterminate results (7/66, 10.6% vs 24/706, 3.4%, p = 0.01). Factors associated with a positive IGRA on multivariable analysis include age (adjusted OR 1.03, 95% CI 1.00–1.06, p = 0.02), IDU (adjusted OR 2.44, 95% CI 1.31–4.55, p = 0.005), a past history of TB disease (adjusted OR 5.44, 95% CI 2.06–14.36, p = 0.001) and past exposure to TB (adjusted OR 2.77, 95% CI 1.11–6.89, p = 0.03) are shown in

Table 1. Baseline Characteristics of Participants (N = 772).

Characteristics		
Mean age ± SD (years)	36.8 ± 9.0	
(range)	(20.0–77.2)	
Men	744	(96.4)
Median duration of HIV diagnosis (years)	1	(0–3)
Baseline CD4 cell count (cells/μL)		
< 200	66	(8.5)
200–349	161	(20.9)
≥ 350	545	(70.6)
HIV-1 RNA viral load (copies/mL)		
< 10,000	597	(77.3)
10,000–99,999	161	(20.9)
≥ 100,000	14	(1.8)
Log HIV-1 RNA viral load (copies/mL)	3.40	(1.74–3.97)
Use of HAART at entry	254	(32.9)
Use of HAART anytime during the study	478	(61.9)
BCG vaccination	755	(97.8)
Past history of TB	40	(5.2)
Past exposure to TB	35	(4.5)

Data are described as n (%), mean (SD, range) or median (IQR). BCG, Bacille-Calmette Guérin; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; IDU, injection drug users; IQR, interquartile range; MSM, men-who-have-sex-with-men; TB, tuberculosis.

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Table 2. CD4 cell counts and HIV-1 RNA viral load were not significant. None of the IGRA-positive participants accepted the offer of IPT.

Incidence and Risk Factors of Developing Active TB Disease

Seventeen incident TB cases (2.2%, 17/772) occurred during 3842 person-years of follow up (0.44 per 100 person-years). The median duration of follow-up was 5.21 years (IQR 4.14–5.81) (Fig 2). Five (29.4%) had extrapulmonary TB, including pleuritis (1), peritonitis (1), meningitis (1), cutaneous (1), and disseminated (1). The diagnosis of TB was established by culture in 8 and a positive TB-PCR of the sputum in 2 (10/17, 59%), pathology in 2 (12%) and by typical clinical or radiologic findings in the remaining 5 (29%).

The median CD4 cell count of the 17 active TB cases at diagnosis was 365 cells/μL (IQR 241–640). At study entry, eight (8/17) were not eligible for ART according to guidelines at the time of the study (i.e. CD4 cells ≥350 cells/μL). Of the 9 patients eligible for ART at study entry, 5 were on ART already, 1 was placed on ART after study entry, 1 refused ART, and 2 were lost to follow-up during the study until the onset of TB.

Univariate Cox regression analysis shows that a baseline CD4 cell count of 200–349 cells/μL, < 200 cells/μL, a positive baseline IGRA, and a baseline HIV viral load ≥ 100,000 copies/mL were associated with a 2-fold, 3-fold, 4-fold, and 10-fold increase in risk for incident active TB, respectively (Table 3), though not reaching statistical significance for CD4 cell count. Multivariable Cox regression analysis identified a positive IGRA (adjusted hazard ratio [HR] 5.04, 95% CI 1.76–14.46, p = 0.003) and an HIV viral load ≥ 100,000 copies/mL (adjusted HR 8.75, 95% CI 1.22–62.56, p = 0.03) (Table 3) as independent risk factors for incident active TB, after adjusting for age, CD4 cell count and HIV viral load.

Table 2. Factors Associated with a Positive Interferon-Gamma Release Assay (IGRA) Result in 741 Participants*.

Variables	Crude OR	(95% CI)	*P* Value	Adjusted OR†	(95% CI)	*P* Value
Age ‡ (years)	1.02	(1.00–1.05)	0.04	1.03	(1.00–1.06)	0.02
Sex, men	0.38	(0.15–0.92)	0.03	039	(0.16–0.99)	0.05
HIV risk factor						
Sexual transmission	1.00			1.00		
IDU	1.71	(1.02–2.89)	0.04	2.44	(1.31–4.55)	0.005
CD4 cell count (cells/μL)						
< 200	0.59	(0.23–1.52)	0.27	0.53	(0.18–1.56)	0.25
200–349	0.59	(0.32–1.11)	0.10	0.55	(0.29–1.05)	0.07
≥ 350	1.00			1.00		
HIV-1 RNA viral load (copies/mL)						
< 10,000	1.00			1.00		
10,000–99,999	0.73	(0.41–1.31)	0.29	0.74	(0.41–1,35)	0.32
≥ 100,000	0.56	(0.07–4.39)	0.58	1.25	(0.13–11.85)	0.84
BCG vaccination	1.04	(0.23–4.61)	0.96	1.26	(0.27–5.79)	0.77
Past history of TB disease	2.78	(1.25–6.17)	0.01	5.44	(2.06–14.36)	0.001
Past exposure to TB	2.78	(1.25–6.17)	0.01	2.77	(1.11–6.89)	0.03

BCG, Bacille-Calmette Guérin; CI, confidence interval; HIV, human immunodeficiency virus; IDU, injection drug users. OR, odds ratio; TB, tuberculosis

*Excluding 31 subjects with indeterminate interferon-gamma release assay result.

†Adjusted for age, sex, HIV risk factor, CD4 cell counts and a past history of TB disease.

‡Test for trend for age: OR 1.03, 95% CI 1.01–1.06, p = 0.02 (adjusted for sex, HIV risk factor, CD4+ T cell count and past TB disease).

doi:10.1371/journal.pone.0135801.t002

Sensitivity and Predictive Values of IGRA

HIV-infected patients with a positive IGRA had a cumulative risk for developing active TB of 6.7% (6/90), significantly higher than the 1.5% (10/651) of those with a negative IGRA (log rank test, p = 0.003) (Fig 3). The negative predictive value was 98.5% (641/651). However, IGRA alone identified only 37.5% (6/17) of patients who developed incident active TB during follow-up.

Algorithm to Predict Risk of Active TB in HIV-Infected Persons

We constructed composite risk factors using baseline CD4 cell count, plasma HIV viral load, and IGRA results. A composite factor of high HIV viral loads ≥ 100,000 copies/mL or low CD4 cell counts of < 350 cells/μL (adjusted HR 4.7, 95% CI 1.49–15.90, p = 0.009) and a positive IGRA in those lacking the composite factor (adjusted HR 6.09, 95% CI 1.52–24.40, p = 0.01) were two independent risk factors for developing incident active TB (Table 3 and Fig 4).

Based on the above findings, we developed a multivariable clinical algorithm (Fig 5): (1) HIV-infected persons with a CD4 cell count < 350 cells/μL or an HIV viral load ≥ 100,000 copies/mL are at a high risk of developing active TB early (pVL/CD4 approach), and should be

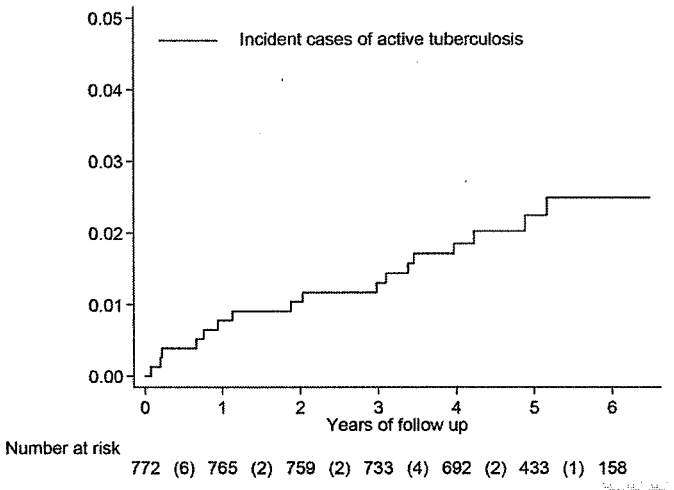


Fig 2. Cumulative incidence of active tuberculosis disease over 5 years in a cohort of HIV-infected adults (N = 772).

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offered IPT; (2) those without these two risk factors, but tested positive for IGRA should also be prioritized for IPT (pVL/CD4 approach augmented by IGRA).

Comparison of Study Algorithm with Alternative Approaches

Compared with an untargeted IPT strategy (WHO approach), the study algorithm targets only HIV-infected persons at high risk for developing incident TB (38.9%, 300/772), and thus avoids unnecessary IPT in 60.6% (468/772) (Table 3). The number needed to treat (NNT) to prevent one case of active TB is reduced from 45 to 23 (Table 4).

Compared with the IGRA-alone strategy, the study algorithm improves the sensitivity to predict the occurrence of incident active TB from 37.5% (6/17) to 76.5% (13/17), and the negative predictive value from 98.5% (641/651) to 99.2% (468/472).

Validation of the Study Algorithm

Active TB developed in 4 of 912 HIV-infected patients in validation cohort 1 (0.17 per 100 person-years) with 40.6% on antiretroviral therapy at study entry [23]and in one of 543 HIV-infected patients in validation cohort 2 (0.60 per 100 person-years) with 90.5% on ART [24]. The area under the ROC curve was 0.692 (95% CI: 0.587–0.798) in our study cohort and 0.792 (95% CI: 0.776–0.808) in the validation cohort 1 and 0.766 in validation cohort 2, respectively. Active TB developed in 2/309 (0.7%) of HIV-infected individuals with low CD4 cell count (< 350/μL) and high HIV viral load (> = 100,000 copies/mL) in cohort 1. For those with high CD4 cell counts (> = 350 cells/μL) and low HIV viral load (< 100,000 copies/mL), active TB developed in 2/72 (2.8%) and 1/45 (2.2%) of HIV-infected individuals testing positive for T-SPOT.TB, in cohort 1 and 2, respectively. None of the patients with negative or indeterminate results developed active TB in the low risk category with high CD4 cell counts and low HIV viral loads.

Discussion

Our results demonstrate that a validated, clinical algorithm can identify those HIV patients at high risk for developing active TB. First, those with a CD4 cell count < 350 cells/μL or an HIV

Table 3. Incident Rate and Risk Factors for Development of Incident Active Tuberculosis Disease in HIV-Infected Persons Using A Composite Risk Factor of High HIV Viral Load, Low CD4 Cell Count and IGRA Positivity (*n* = 772).

						Model 1			Model 2 (composite risk factor)		
Variables	**Incidence rate†**	**(95% CI)**	**Crude HR**	**(95% CI)**	**P value**	**Adjusted HR***	**(95% CI)**	**P value**	**Adjusted HR****	**(95% CI)**	***P* Value**
Age (years)											
< 30	0.20	(0.05–0.79)	1.00			1.00			1.00		
30–39	0.48	(0.24–0.96)	2.40	(0.51–11.28)	0.27	2.41	(0.51–11.36)	0.27	2.28	(0.48–10.76)	0.30
≥ 40	0.61	(0.29–1.27)	3.02	(0.63–14.52)	0.17	3.12	(0.64–15.21)	0.16	2.54	(0.53–12.27)	0.25
Sex											
male	0.46	(0.28–0.74)	1.00			—			—		
Female	0.00		—			—			—		
HIV risk factor											
MSM	0.61	(0.25–1.47)	1.00			1.00			1.00		
Heterosexual	0.73	(0.18–2.91)	1.19	(0.23–6.13)	0.84	1.10	(0.21–5.78)	0.91	1.29	(0.25–6.67)	0.76
IDU	0.37	(0.20–0.68)	0.63	(0.22–1.86)	0.41	0.78	(0.23–2.62)	0.68	0.73	(0.24–2.20)	0.57
BCG vaccination:											
No	0.00		—			—			—		
Yes	0.45	(0.28–0.73)	—			—			—		
Past history of TB:											
No	0.44	(0.27–0.71)	1.00			1.00			1.00		
Yes	0.58	(0.08–4.10)	1.28	(0.17–9.63)	0.81	1.00	(0.12–8.30)	1.00	0.82	(0.11–6.24)	
Past exposure to TB:											
No	0.43	(0.26–0.70)	1.00			1.00			1.00		
Yes	0.75	(0.11–5.32)	1.66	(0.22–12.59)	0.63	1.24	(0.16–9.72)	0.84	1.19	(0.16–9.06)	
CD4 cell counts (cells/μl)											
< 200	1.05	(0.34–3.27)	3.18	(0.86–11.79)	0.08	1.46	(0.27–7.79)	0.66	—		
200–349	0.66	(0.27–1.59)	2.03	(0.68–6.06)	0.21	1.78	(0.59–5.39)	0.31	—		
≥ 350	0.32	(0.17–0.62)	1.00			1.00			—		
HIV-1 RNA viral load (copies/ml)											
< 10,000	0.34	(0.18–0.63)	1.00			1.00			—		
10,000–99,999	0.60	(0.25–1.44)	1.79	(0.61–5.23)	0.29	1.70	(0.58–5.01)	0.34	—		
≥ 100,000	3.43	(0.86–13.73)	9.87	(2.15–45.29)	0.003	8.75	(1.22–62.56)	0.03	—		

(*Continued*)

Table 3. (*Continued*)

Variables	Incidence rate†	(95% CI)	Crude HR	(95% CI)	P value	Model 1 Adjusted HR*	(95% CI)	P value	Model 2 (composite risk factor) Adjusted HR**	(95% CI)	*P* Value
Interferon-gamma release assay (IGRA)											
Positive	1.30	(0.58–2.89)	4.18	(1.52–11.52)	0.006	5.04	(1.76–14.46)	0.003	—		
Indeterminate	0.69	(0.10–4.89)	2.16	(0.28–16.87)	0.46	1.66	(0.21–13.38)	0.63	—		
Negative	0.31	(0.17–0.57)	1.00			1.00			—		
Composite risk factor											
pVL < 100,000 copies/mL, CD4 ≥ 350 cells/μL, andIGRA negative or indeterminate	0.17	(0.06–0.44)	1.00			—			1.00		
pVL < 100,000 copies/mL, CD4 ≥ 350 cells/μL, and IGRA positive	1.05	(0.39–2.79)	6.28	(1.57–25.13)	0.01	—			6.09	(1.52–24.40)	0.01
pVL ≥ 100,000 copies/mL or CD4 < 350 cells/μL	0.86	(0.45–1.66)	5.10	(0.57–16.60)	0.01	—			4.87	(1.49–15.90)	0.01

BCG, Bacille-Calmette Guérin; CI, confidence interval; HR, hazard ratio; HIV, human immunodeficiency virus; IDU, injection drug users; IGRA, interferon-gamma release assay; MSM, men-who-have-sex-with-men; TB, tuberculosis; pVL, plasma HIV-1 RNA viral load.

*Model 1: Adjusted for age, CD4 cell counts and HIV viral load

**Model 2: Adjusted for age and the composite risk factor of CD4 cell count, HIV viral load, or QuantiFERON.

† per 100 person-years

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viral load ≥ 100,000 copies/mL are at high risk of TB, and should be offered IPT regardless of IGRA results. Second, for those with a CD4 cell count ≥ 350 cells/μL and an HIV viral load < 100,000 copies/mL, IGRA can identify those at high risk of TB who may benefit from IPT. The accuracy of predicting risk of TB is markedly improved by incorporating CD4 cell

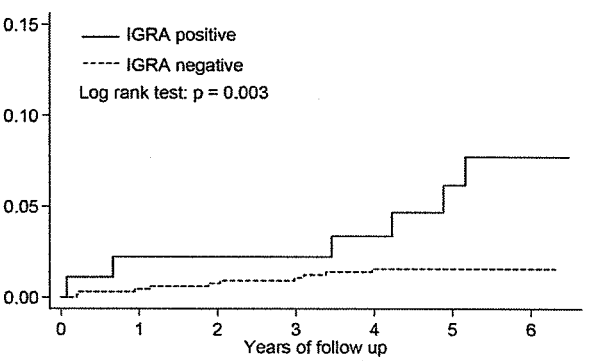


Fig 3. Kaplan-Meier curve of HIV-infected patients who developed active tuberculosis disease by interferon-gamma release assay (IGRA) status, excluding 31 subjects with indeterminate results (N = 741).

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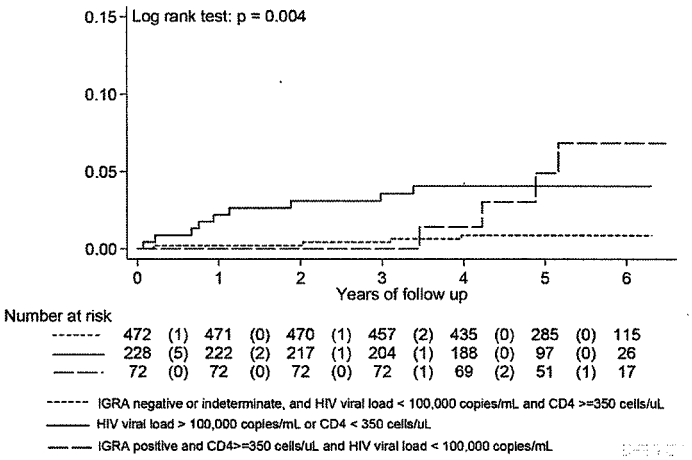


Fig 4. Kaplan-Meier curve to development of active tuberculosis disease by composite risk factor combining high HIV viral load of greater than 100,000 copies per mL, CD4 cell count and interferon-gamma release assay (IGRA)(N = 772).

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counts and HIV viral loads into the algorithm. Compared with an IGRA alone approach, the sensitivity was improved from 37.5% to 76.5%; the negative predictive value was improved from 98.5% to 99.2%. Targeting IPT on the high risk group identified by this algorithm will spare 60.6% of the HIV patients from unnecessary treatment compared to untargeted strategy.

The study algorithm was validated in 2 separate, HIV-infected cohorts using T-SPOT.TB as IGRA, from Northern Taiwan [23–24]. Validation using these cohorts found a greater area under the ROC than the study algorithm. Similar to our study (17, 2.2%), a low number of cases of active TB (5, 0.6% and 1, 1.6%) was observed in both validation cohorts. The overall incidence of active TB was similar in our study cohort (0.44 per 100 person-years) compared to the validation cohorts, with an overall incidence rate of 0.17 per 100 person-years [23] in cohort 1 and 0.60 per 100 person-years in cohort 2 [24]. Our results and algorithm validates

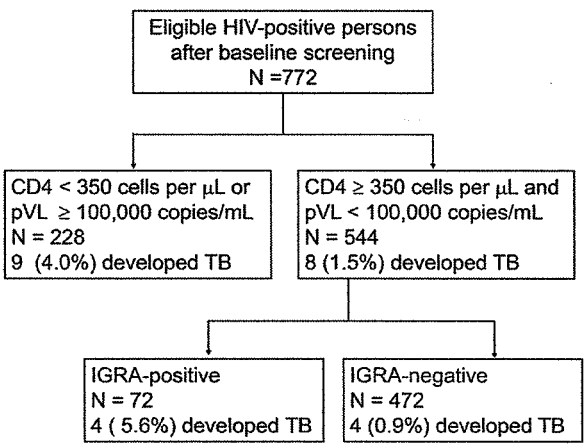


Fig 5. A multivariable algorithm to predict risk of active tuberculosis in people with HIV infection using a composite risk factor of HIV viral load, CD4 cell count and interferon-gamma release assay (IGRA) result.

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Table 4. Comparison of Different Approaches to Prevent Tuberculosis in People Living With HIV.

Variable	Study Algorithm*(N = 772)		IGRA-alone Approach*	WHO Approach*
	pVL/CD4 approach†	pVL/CD4 approach augmented by IGRA**	(N = 772)	(N = 772)
Isoniazid preventive treatment needed	228 (29.5)	300 (38.9)	90 (11.7)	772 (100.0)
IGRA testing needed	0	544	772	0
HIV viral load needed	772	772	0	0
CD4 cell count needed	772	772	0	0
False-negative results	8 (1.5)	4 (0.8)	11 (1.6)	0 (0.0)
True positives (Sensitivity)	9 (52.9)	13 (76.5)	6 (35.3)	17 (100.0)
Patient unnecessarily treated	219 (28.4)	287 (31.2)	84 (10.9)	755 (97.8)
Number needed to treat (NNT) (95% CI)assuming 90% efficacy of IPT	25 (14–69)	23 (14–47)	15 (7–81)	45 (29–81)
Number needed to treat (NNT) (95% CI)assuming 50% efficacy of IPT	51 (39–71)	46 (37–61)	30 (21–54)	91 (79–108)
Characteristics of patients with true positive results selected for treatment				
No of patients	9	13	6	17
Median CD4 cell count at presentation (cells/μl)	272 (39–441)	287 (39–932)	639 (76–932)	365 (39–932)
Median months to onset of tuberculosis	13.3 (2.8–42.5)	24.5 (2.8–64.0)	48.1 (2.8–64.0)	26.3 (2.8–64.0)
Characteristics of patients with false-negative results				
No of patients	8	4	10	—
Median CD4 cell count at presentation (cells/μl)	639 (365–932)	462 (365–902)	337 (39–902)	—
Median months to onset of tuberculosis	46.5 (4.4–64.0)	32.8 (4.4–49.6)	24.5 (4.4–49.6)	—

Data are described as n (%) or median (range), unless otherwise specified. CI, confidence interval. IGRA; interferon-gamma release assay; IQR, interquartile range; pVL, HIV plasma viral load; WHO, World Health Organization.

* pVL/CD4 approach include those with high HIV viral load of > = 100,000 copies/mL and low CD4 cell counts of < 350 cells/μL

**pVL/CD4 approach augmented by IGRA includes those in the pVL/CD4 approach and additionally those with a positive IGRA test; IGRA approach include those with positive IGRA tests only and the W.H.O. approach does no testing and includes all.

†In those with CD4 ≥ 350 cells/μL, the proportion testing IGRA-positive was 72/545 (13.2%), and in those with high HIV plasma viral load ≥ 100,000 copies/mL, 1/14 (7.1%).

doi:10.1371/journal.pone.0135801.t004

previous study cohorts, predicting a higher risk of active TB in those with low CD4 cell counts and high HIV viral loads (2/309, 0.7%), and in those with high CD4 cell counts and low HIV viral loads, only when IGRA was positive (2/72, 2.8% and 1/45, 2.2% in cohort 1 and 2, respectively).

A recent, randomized controlled trial in South Africa, a high TB burden country, showed that isoniazid plus ART was effective in preventing TB, and the effect was not restricted to those with positive TST or IGRA, which led to the recommendation for universal IPT in HIV patients receiving ART [13]. However, the participants of this trial had a median CD4 cell count of 216 cells/μL (range: 152–360). The majority of these patients would be categorized as high TB risk by our clinical algorithm, and benefit from IPT. Whether HIV patients with a low-TB risk defined by our algorithm (i.e. those who had a CD4 cell count ≥ 350 cells/μL, an HIV viral load < 100,000 copies/mL, and a negative IGRA), can benefit from IPT can only be answered by future studies.

Our finding that a composite risk factor of either a low baseline CD4 cell count of < 350 cells/μL or a high HIV viral load ≥ 100,000 copies/mL as an independent predictor for incident

active TB in HIV persons (adjusted HR 4.87) is consistent with existing literature. Previous studies showed that the risk of incident active TB in untreated HIV-infected persons increases to more than 4-fold of the baseline risk when the CD4 cell counts decrease to less than 350 cells/μL [26]. In those receiving HAART, the risk of incident active TB was associated with a low baseline CD4 cell count [2, 6, 27]. In addition to CD4 cell counts of less than 350, a high viral load of > 10,000 copies/mL was shown to be one of the risk factors associated with TB developing after more than six months of HAART [27]. Similarly, this study shows that HIV-infected people with high viral loads of ≥ 100,000 copies/mL are at high risks of developing active TB early, regardless of CD4 cell counts.

Previous studies have shown that false negative and indeterminate results on IGRA in HIV-infected persons are associated with low CD4 cell counts, as was seen in our analysis [16, 28]. In our study, 31 patients (4%) had an indeterminate IGRA result, a group with significantly lower CD4 cell counts than those with either a positive or negative IGRA. However, a positive IGRA test in HIV-infected persons with relatively intact immunity (CD4 cell count > 350 cells/μL and HIV viral load < 100,000 copies/mL) can improve the estimation for the risk of incident active TB, and increases the sensitivity from 52.9% (HIV viral load and CD4 approach alone) to 76.5% (HIV viral load and CD4 approach augmented with IGRA).

The positive predictive value (6.5%) and negative predictive value (98.5%) of IGRA for active TB in our study are similar to previously reported data [29–35]. A recent meta-analysis found IGRA to have a pooled positive predictive value of 8.5% (55/648) in healthy persons in contact with active TB cases, and 6.0% (11/182) in 4 HIV studies [31]; but only two of these studies were designed to address the ability of IGRA to identify HIV-infected individuals likely to progress to incident active TB [35–36]. The first study was conducted in Austria, a country with low TB incidence, and found that 8.3% (3/36) of HIV-infected persons with a positive IGRA developed TB disease within 19 months of follow up, with a negative predictive value of 100% (738/738)[35]. The second study, conducted in Kenya, a high TB incidence country, which found that IGRA had a positive predictive value of 5.5% (6/110) among HIV-infected pregnant women during a mean follow up of 1.28 years, with a negative predictive value of 98.0% (145/148)[36].

The overall incidence of active TB in our study (2.2%, 0.44 per 100 person-years) was higher than that observed in Austria, a low TB transmission country (0.4%),[35] but much lower than the 2.7 per 100 person-years observed in Kenya, a high TB transmission country [36]. The incidence of active TB in those with a positive IGRA in the present study (1.30 per 100 person-years) was 70% lower than that observed in Kenya (4.2 per 100 person-years)[36]. The difference in active TB incidence is most likely explained by a difference in ART coverage between the two countries. The free provision of ART to all HIV-infected patients in Taiwan is an important protecting factor against TB in the present study.

None of the participants tested IGRA positive chose to receive IPT. Taiwan CDC has a policy endorsing the use of IPT in HIV-infected patients, but similar to many countries worldwide [19], uptake has been limited due to lack of programs to implement the policy [24], perceived low risk of active TB [24], and concerns with INH hepatoxicity, which has an overall case-fatality rate of 10% in those with clinically apparent hepatitis. Tuberculin skin test is not provided for testing to adults due to high coverage of neonatal BCG vaccination in Taiwan since 1965, with booster doses given to school children until 1997 [23]. Currently in Taiwan, IPT is only offered to children under contact investigations since 2008 and to young adults born before 1987, as of April 2012 [22].

IGRA is not readily available and is not reimbursed by the national health insurance for testing. This led to an extremely low uptake of latent TB infection testing and treatment among HIV patients in Taiwan, which, was accepted as the standard of care during the study period

(2006–2010) in Taiwan. The free provision of ART for HIV-infected patients eligible for treatment provided very effective protection against TB. The additional protective effect of IPT in HIV-infected patients on ART was suggested by retrospective cohort studies [19, 29, 37] and was not established until 2014, when a randomized control trial of isoniazid plus ART to prevent TB in high TB burden country was published [13]. The risk of active TB is 11 times higher than the general population, even in a cohort with 90% ART coverage [24]. Our clinical algorithm may provide a tool for programs to implement policy, and facilitate increase uptake of IPT in addition to ART in countries with intermediate TB burden.

TSPOT.TB uses the same antigens specific for QuantiFERON-TB Gold test used in this study (ESAT-6 and CFP-10), thus theoretically the results of this study could be generalizable to T-SPOT.TB. Although studies comparing the 2 IGRAs (T-SPOT.TB and QFT) in HIV-infected individuals have shown concordance which varied between poor (kappa = 0.15)[38] to fair (kappa = 0.35)[39] to moderate (kappa = 0.56)[40], validation of this clinical algorithm in 2 separate HIV-infected cohorts in Northern Taiwan using T-SPOT.TB suggests that the conclusions of this study can be extended to T-SPOT.TB.

Our study has several limitations. Firstly, the routine provision of free HAART to all participants with CD4 cell counts of less than 350 cells/μL, according to treatment guidelines, precluded the ability of our study to demonstrate the protective effect of HAART on reducing the risk of incident active TB, due to lack of a comparison group who did not receive HAART. Secondly, we did not evaluate the potential usefulness of TST when combined with CD4 cell count and HIV viral load in stratifying risk for active TB, because cutaneous anergy or false negative TSTs may occur in up to 60% of HIV-infected patients [16–17], and routine BCG vaccination in Taiwan renders TST difficult to interpret due to false positivity. However, TST was shown to be more specific than IGRA in a recent study done in college students with low or no risk for TB [41], and may be incorporated into an algorithm approach in settings where BCG vaccination is not routinely given. Thirdly, generalizability to females is cautioned as almost all study participants were male. Finally, this study was conducted in an economically developed country of moderate TB incidence, where HAART and advanced medical care, including CD4 cell counts, HIV viral loads and IGRA, are available for HIV-infected patients. Our findings therefore may not be generalizable to those settings where medical resources are constrained or with very high TB burdens.

In conclusion, a clinical algorithm incorporating CD4 cell count, HIV viral load, and IGRA status can identify those HIV patients at high risk of incident active TB who are most likely to benefit from IPT. This algorithm can be used to guide targeted TB preventive treatment in PLHIV living in low-to-moderate TB burden settings, while avoiding unnecessary exposure of low-risk patients to drug toxicity and reduce the burden on the healthcare system.

Supporting Information

S1 Dataset.
(XLS)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: SSL HL HT IS KW CS JC YC CF. Performed the experiments: SSL HL HT IS KW CS JC YC CF. Analyzed the data: SSL HL HT IS KW CS JC

YC CF. Contributed reagents/materials/analysis tools: SSL HL HT IS KW CS JC YC CF CY HS CH. Wrote the paper: SSL HL HT IS KW CS JC YC CF.

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SYNOPSIS

Mycobacterium abscessus Complex Infections in Humans

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Release date: August 13, 2015; Expiration date: August 13, 2016

Learning Objectives

Upon completion of this activity, participants will be able to:

- Describe clinical and nosocomial aspects of *M. abscessus* infections, based on a literature review
- Compare clinical and treatment aspects of infections with *M. abscessus* subspecies
- Describe treatment of *M. abscessus* infections

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Mycobacterium abscessus complex comprises a group of rapidly growing, multidrug-resistant, nontuberculous mycobacteria that are responsible for a wide spectrum of skin and soft tissue diseases, central nervous system infections, bacteremia, and ocular and other infections. *M. abscessus* complex is differentiated into 3 subspecies: *M. abscessus* subsp. *abscessus, M. abscessus* subsp. *massiliense*, and *M. abscessus* subsp. *bolletii*. The 2 major subspecies, *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense*, have different *erm*(41) gene patterns. This gene provides intrinsic resistance to macrolides, so the different patterns lead to different treatment outcomes. *M. abscessus* complex outbreaks associated with cosmetic procedures and nosocomial transmissions are not uncommon. Clarithromycin, amikacin, and cefoxitin are the current antimicrobial drugs of choice for treatment. However, new treatment regimens are urgently needed, as are rapid and inexpensive identification methods and measures to contain nosocomial transmission and outbreaks.

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Mycobacteria are divided into 2 major groups for the purpose of diagnosis and treatment: *Mycobacterium tuberculosis* complex, which comprises *M. tuberculosis,* and nontuberculous mycobacteria (NTM), which comprise all of the other mycobacteria species that do not cause tuberculosis. NTM can cause pulmonary disease resembling tuberculosis, skin and soft tissue infections (SSTIs), central nervous system infections, bacteremia, and ocular and other infections (*1*,*2*). Over the past decade, the number of NTM disease cases worldwide has markedly increased (*3*,*4*), and the upsurge cannot be explained solely by increased awareness among physicians and advances in laboratory methods (*3*).

M. abscessus complex is a group of rapidly growing, multidrug-resistant NTM species that are ubiquitous in soil and water (*1*). Species comprising *M. avium* complex (MAC) are the most common NTM species responsible for disease; however, infections caused by *M. abscessus* complex are more difficult to treat because of antimicrobial drug resistance (*5*). *M. abscessus* complex is also resistant to disinfectants and, therefore, can cause postsurgical and postprocedural infections (*2*,*5*). Although *M. abscessus* complex most commonly causes SSTIs and pulmonary infections, the complex can also cause disease in almost all human organs (*2*,*5*). To improve our understanding of *M. abscessus* complex infections, we reviewed the epidemiology and clinical features of and treatment and prevention measure for diseases caused by the organisms as well as the taxonomy and antimicrobial susceptibilities of these organisms.

Search Strategy and Selection Criteria

We performed a PubMed search for *M. abscessus* complex articles published during January 1990–December 2014, using the following search terms: *M. abscessus*, *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *bolletii*, *M. abscessus* subsp. *massiliense*, *M. massiliense*, *M. bolletii*, and nontuberculous mycobacteria. Only articles published with abstracts in English were selected.

Taxonomy and Epidemiology

Bacterial Classification

M. abscessus was first isolated from a knee abscess in 1952 (*1*). *M. abscessus* and *M. chelonae* were originally considered to belong to the same species ("*M. chelonei*" or "*M. chelonae*"), but in 1992, *M. abscessus* was reclassified as an individual species (*1*). After *M. abscessus* was recognized as an independent species, new subspecies, including *M. massiliense* and *M. bolletii*, were discovered. Debate has ensued over whether *M. massiliense* and *M. bolletii* should be reunited to form one subspecies, *M. abscessus* subsp. *bolletii* (*6*). It is hoped that the debate will be settled as a result of findings from several recent studies that clearly demonstrated, by genome comparison, that *M. abscessus* complex comprises 3 entities: *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense*, and *M. abscessus* subsp. *bolletii* (*7*–*11*). Serial changes in the taxonomic classification and nomenclature of *M. abscessus* complex, from 1992 to 2013, are shown in Figure 1.

M. abscessus subsp. *bolletii* is recognized as a rare pathogen with a functional inducible erythromycin ribosome methyltransferase (*erm*) (41) gene. In most *M. abscessus* subsp. *abscessus* mycobacterium, this gene leads to macrolide resistance. *M. abscessus* subsp. *massiliense* has been proposed to have a nonfunctional *erm*(41) gene, leading to macrolide susceptibility and a favorable treatment outcome for infections (*7*–*11*).

Laboratory Identification

Definitive diagnosis of *M. abscessus* complex infection in humans is invariably determined by the isolation of *M. abscessus* complex from clinical specimens. The correct subspecies identification of *M. abscessus* complex has traditionally relied on phenotypic methods (e.g., biochemical testing for the utilization of citrate) to distinguish them from closely related species like *M. chelonae* (*1*). However, this method is not accurate enough to differentiate between the 2 main subspecies of the complex. Instead,

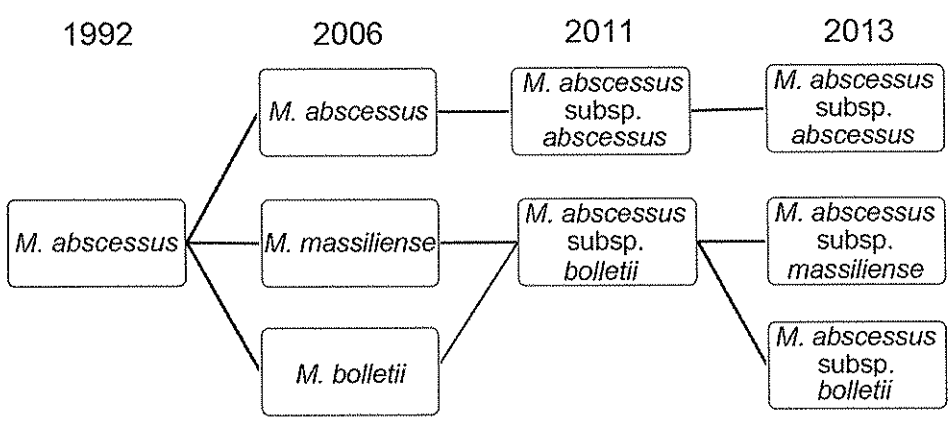


Figure 1. Serial changes in the nomenclature and taxonomic classification of *Mycobacterium abscessus* complex, 1992–2013.

rpoB gene–based sequencing is a more reliable method for correctly identifying *M. abscessus* complex to the subspecies level (*10*). However, because of the limited differences between the subspecies of *M. abscessus* complex, some researchers have questioned the accuracy of identification results from the sequencing of a single gene, especially the *rpoB* gene (*10*). Many schemes have been used in an attempt to accurately differentiate between subspecies, such as multilocus gene sequence typing, sequencing of the *erm* gene, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Figure 2) (*10*,*12*). Nonetheless, because the taxonomic classification is still changing, the debate over the optimal identification method will probably also continue.

Disease Burden

The global isolation and epidemiology of *M. abscessus* complex are diverse. Furthermore, due to limitations in correct and detailed species identification, previous epidemiologic studies often referred to *M. abscessus* complex as *M. chelonae/abscessus* group or rapidly growing mycobacteria (*13*). In the United States, *M. abscessus/chelonae* complex infections are secondary only to MAC infections, compromising 2.6%–13.0% of all mycobacterial pulmonary infections across various study sites. This percentage correlates to an annual prevalence of <1 *M. abscessus/chelonae* pulmonary infections per 100,000 population, but the prevalence is increasing (*13*). *M. abscessus* complex is especially prevalent in East Asia. For example, in Taiwan, *M. abscessus* complex comprises 17.2% of all clinical NTM isolates, which correlates to 1.7 cases/100,000 population (*4*). According to current studies, the proportion of *M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *abscessus* is about the same among all clinical isolates (*12*). *M. abscessus* subsp. *bolletii* is rarely isolated (*7*).

Clinical Diseases

Respiratory Tract Infections

M. abscessus complex can cause pulmonary disease, especially in vulnerable hosts with underlying structural lung disease, such as cystic fibrosis, bronchiectasis, and prior tuberculosis (*2*). *M. abscessus* complex pulmonary disease usually follows an indolent, but progressive, course, causing persistent symptoms, decline of pulmonary function, and impaired quality of life; however, the disease can also follow a fulminant course with acute respiratory failure (*2*,*14*). Establishing a diagnosis of pulmonary disease due to *M. abscessus* complex is not straightforward because isolation of *M. abscessus* complex from respiratory samples is not, in and of itself, diagnostic of pulmonary disease (*2*). According to guidelines published by the American Thoracic Society/Infectious Diseases Society of America in 2007, the diagnosis of *M. abscessus* complex pulmonary disease requires the fulfillment of clinical and microbiological criteria, such as the presence of clinical symptoms; radiographic evidence of lesions compatible with NTM pulmonary disease; appropriate exclusion of other diseases; and, in most circumstances, positive culture results from at least 2 separate expectorated sputum samples (*2*). Common radiographic findings of *M. abscessus* complex pulmonary infection (i.e., bronchiolitis; bronchiectasis; nodules; consolidation; and, less frequently, cavities) are shown in Figure 3 (*2*).

M. abscessus complex is especially prevalent in respiratory specimens from patients with cystic fibrosis (*7*,*15*). Recent studies have shown that *M. abscessus* complex infection is no longer a contraindication for lung transplantation, although postoperative complications and a prolonged treatment course can be expected (*7*).

Pulmonary disease caused by *M. abscessus* complex is notoriously difficult to treat. Although there is no standard

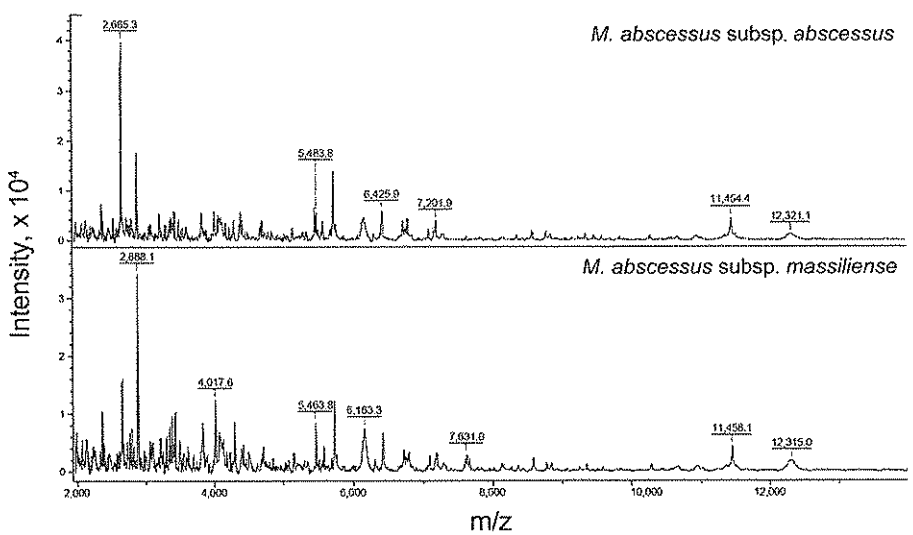


Figure 2. Spectrum of *Mycobacterium abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense* created by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry Biotyper system (Microflex LT; Bruker Daltonik GmbH, Bremen, Germany). The absolute intensities of the ions are shown on the *y*-axis, and the masses (m/z) of the ions are shown on the *x*-axis. The m/z values represent the mass-to-charge ratio.

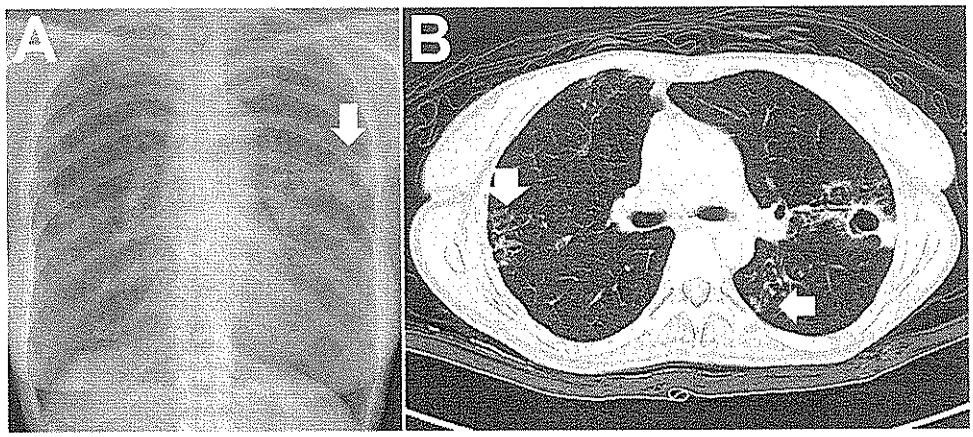


Figure 3. Chest radiograph (A) and computed tomography scan (B) images for a patient with pulmonary disease due to *Mycobacterium abscessus* subsp. *abscessus*. A) The arrow indicates a cavity with surrounding consolidation over the left upper lung. B) Vertical arrow indicates bronchiectasis; horizontal arrow indicates nodules.

treatment, current guidelines suggest the administration of macrolide-based therapy in combination with intravenously administered antimicrobial agents; however, this regimen has been shown to have a substantial cytotoxic effect (*2*). Of 65 patients with pulmonary disease due to *M. abscessus* complex who received an initial 4-week course of intravenous antimicrobial agents followed by macrolide-based combination therapy, 38 (58%) had *M. abscessus*–negative sputum samples >12 months after treatment (*16*). Surgical resection of localized disease in addition to antimicrobial therapy has been shown to elicit a longer microbiologic response than antimicrobial agents alone: sputum samples were *M. abscessus* complex–negative for at least 1 year in 57% versus 28% of these treatment groups, respectively (*17*). According to the 2007 American Thoracic Society/ Infectious Diseases Society of America guidelines, the treatment options remain limited with current antimicrobial agents, and *M. abscessus* complex pulmonary disease is still considered a chronic incurable disease (*2*).

The advancement of subspecies differentiation has allowed for more effective management of pulmonary disease caused by *M. abscessus* complex. For example, unlike *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense* does not have inducible resistance to clarithromycin (*7*). Therefore, knowing that a patient's infection is due to *M. abscessus* subsp. *massiliense* rather than 1 of the other 2 subspecies enables the physician to confidently administer clarithromycin (*7*,*18*). In a large study on treatment outcome in patients with pulmonary disease caused by *M. Abscessus* subsp. *massiliense* or *M. abscessus* subsp. *abscessus*, all patients had similar clinical signs, radiographic findings, and treatment regimens (*18*). However, after treatment, the percentage of patients who had negative sputum culture results was much higher in the *M. abscessus* subsp. *massiliense*–infected group (88%) than in the *M. abscessus* subsp. *abscessus*–infected group (25%) (*18*). The lack of efficacy of clarithromycin-containing antimicrobial therapy against *M. abscessus* subsp. *abscessus* isolates in the study could be explained by the subspecies' inducible resistance to clarithromycin. The study clearly demonstrated how *M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *abscessus* have different susceptibility profiles to combination therapy containing clarithromycin and different outcomes from such treatment (*18*).

SSTIs

SSTIs are also commonly caused by *M. abscessus* complex; infections range from deep tissue infections to localized skin infections. The 2 major mechanisms for acquiring an *M. abscessus* complex–associated SSTI are by 1) direct contact with contaminated material or water through traumatic injury, surgical wound, or environmental exposure and 2) secondary involvement of skin and soft tissue during disseminated disease (*19*). SSTIs caused by *M. abscessus* complex have been reported in patients who recently underwent cosmetic procedures (e.g., mesotherapy), tattooing, and acupuncture (*19*). *M. abscessus* complex SSTIs can also develop after exposure to environmental sources, such as spas and hot springs (*19*,*20*). More often, however, these SSTIs develop among hospitalized postsurgical patients, in whom surgical wound infections are most commonly due to *M. abscessus* subsp. *massiliense* (*21*,*22*). Disseminated *M. abscessus* complex infections with skin and soft tissue involvement also commonly occur (*23*). Of note, however, the presence of *M. abscessus* complex SSTIs can result in or from disseminated *M. abscessus* complex infections (*23*). *M. abscessus* complex skin infection have diverse presentations, including cutaneous nodules (usually tender), erythematous papules/pustules, and papular eruptions or abscesses (Figure 4) (*19*).

Central Nervous System Infections

Central nervous system (CNS) infections caused by *M. abscessus* complex are rare, but when they do occur, meningitis and cerebral abscesses are the most common manifestations (Figure 5). Although MAC is responsible for most

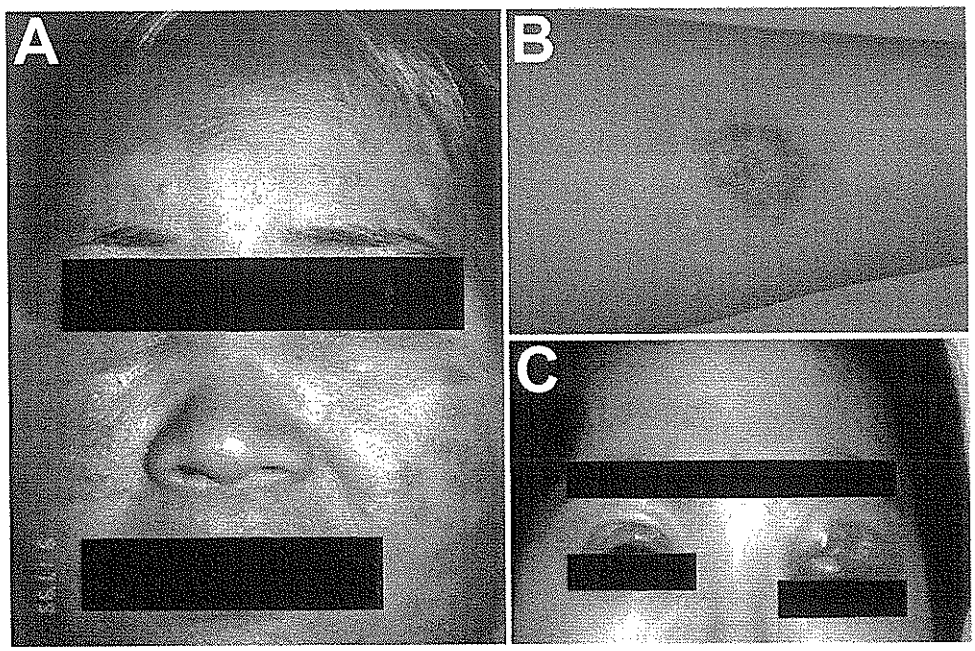


Figure 4. Skin lesions caused by *Mycobacterium abscessus* subsp. *abscessus*. A) Diffuse erythematous papular eruptions on the face and bilateral cervical lymphadenitis in a middle-aged man. B) A circumscribed subcutaneous nodule with pus discharge on the right arm of a 12-year-old boy. C) Wound infection over both upper eyelids of a 36-year-old woman; the infection developed 1 week after cosmetic surgery.

NTM CNS infections, especially in HIV-infected hosts, *M. abscessus* complex has increasingly been reported to cause CNS infections in HIV-negative patients (*21*). In one study, *M. abscessus* was responsible for most NTM CNS infections in HIV-seronegative patients (8/11 patients), especially in patients who had undergone neurosurgical procedures, patients who had intracranial catheters, and patients with otologic diseases. Treatment outcome depended on the patient's underlying disease and health status. Clarithromycin-based combination therapy for at least 1 year plus surgical intervention, if needed, offered the best chance for cure (*21*).

Disseminated Diseases and Bacteremia

Disseminated *M. abscessus* complex infections, such as lymphadenopathy, SSTIs, pulmonary infections, and bacteremia, are on the rise (*23*), and bacteremia caused by *M. abscessus* complex is most often associated with catheter use (*24*,*25*). A recent study showed that surgical wound infection may be the portal of entry, especially for *M. abscessus* subsp. *massiliense* (*26*). Optimal treatment modalities include removal of intravascular catheters, surgical debridement, and administration of intravenous antimicrobial agents chosen on the basis of drug susceptibility test results.

Disseminated *M. abscessus* complex infections tend to occur in immunocompromised hosts, including persons with HIV. However, these infections can also occur in HIV-negative patients. Browne et al. (23) recently showed that neutralizing anti–interferon-γ autoantibodies were present in 81% of HIV-negative patients with disseminated NTM-associated infections, and in adults, these antibodies were associated with adult-onset immunodeficiency similar to that seen in advanced HIV infection. This adult-onset immunodeficiency status can lead to disseminated NTM disease that mimics advanced HIV infection (*23*).

Ocular Infections

The incidence of NTM ocular infections (keratitis, endophthalmitis, scleritis, and other tissues of the ocular area) has increased over the past decade, and the increase has been attributed to the *M. chelonae/abscessus* group (*27*). Interpreting the real trend in ocular infections caused by *M. abscessus* complex is difficult because most studies have not used reliable tests to differentiate between *M. abscessus* complex and *M. chelonae* (*27*).

Initial treatment of *M. abscessus* complex ocular infections involves the discontinuation of topical corticosteroids, if used. The optimal treatment strategy (topical therapy, systemic antimicrobial agents, and surgical intervention) depends on the site of the ocular infection (*28*). Topical therapy, particularly topical amikacin and clarithromycin, can be used to treat some *M. abscessus* complex ocular infections (e.g., conjunctivitis, scleritis, keratitis, endophthalmitis) (*28*), and systemic antimicrobial agents can be used for all ocular infections (*28*). Surgical debridement, including removal of infected tissue, should be considered and is necessary for treatment of infections in some patients (*28*). Treatment outcome varies according to the site of infection, and early recognition of the infection is crucial.

Nosocomial Outbreaks and Transmission

Outbreaks of *M. abscessus* complex infections in hospital and clinic settings have been reported worldwide (*19*). Many of the outbreak events occur in clinics conducting cosmetic surgery, liposuction, mesotherapy, or intravenous

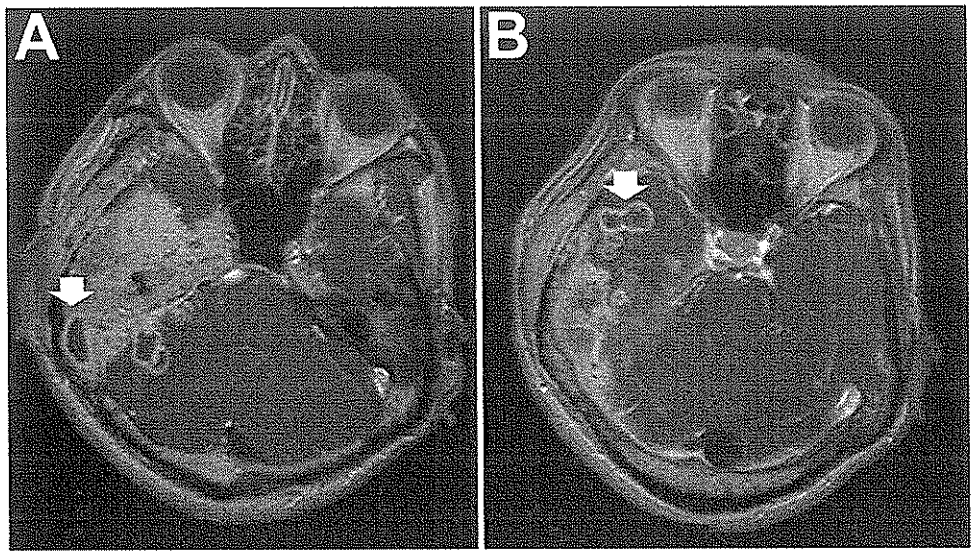


Figure 5. Brain computed tomography scan images for a patient with central nervous system infection caused by *Mycobacterium abscessus* subsp. *bolletii*. Arrows indicate abnormal nodular pachymeningeal thickening and leptomeningeal and intraparenchymal extension with multiple rim-enhancing lesions in the right cerebellum (A) and right temporal lobe (B), indicating cerebral abscesses.

infusion of cell therapy (*29*). Proposed sources of transmission include contaminated disinfectants, saline, and surgical instruments as well as contact transmission between patients (*19*,*30*,*31*).

M. abscessus complex transmission involves vulnerable hosts and causes substantial illness and death; thus, concern is also rising regarding outbreaks in centers specializing in lung transplantation and treatment of cystic fibrosis (*30*). Whole-genome sequencing of outbreak isolates has provided evidence of patient-to-patient transmission of *M. abscessus* complex; this transmission is most likely indirect rather than direct (*30*).

Antimycobacterial Susceptibilities

M. abscessus complex is notoriously resistant to standard antituberculous agents and most antimicrobial agents (*5*). The Clinical and Laboratory Standards Institute recommends testing rapidly growing mycobacteria for susceptibility to macrolides (clarithromycin and amikacin), aminoglycosides, fluoroquinolones, imipenem, doxycycline, tigecycline, cefoxitin, cotrimoxazole, and linezolid (*32*). The recommended drug susceptibility testing method is broth microdilution in cation-adjusted Mueller-Hinton broth supplemented with oleic albumin dextrose catalase (*32*). Among the agents suggested for *M. abscessus* complex susceptibility testing, clarithromycin, amikacin, and cefoxitin have the best in vitro antimycobacterial activity (*7*,*32*,*33*).

Recent major studies presenting susceptibility and resistance rates for *M. abscessus* subsp. *massiliense*, *M. abscessus* subsp. *abscessus*, and *M. abscessus* complex against 7 antimicrobial agents are summarized in Table 1. Most of the studies are from Asia, and the resistance rate for clarithromycin ranges from 0 to 38%. The resistance rates for cefoxitin (overall 15.1%) and amikacin (overall 7.7%) are also low. Doxycycline, quinolones (including moxifloxacin and ciprofloxacin), and imipenem had high resistance rates. Therefore, local susceptibility data are needed to guide treatment.

Because of its rarity, *M. abscessus* subsp. *bolletii* is discussed separately here. These mycobacteria are uniformly resistant to drugs recommended for use against *M. abscessus* complex. In one study, high MICs of tested antimycobacterial agents were observed, and amikacin probably had the highest activity (i.e., the lowest MIC) (*33*).

Recent studies have reported on the importance of the *erm*(41) gene in *M. abscessus* complex; this gene confers macrolide resistance through methylation of 23S ribosomal RNA (*39*). The *erm*(41) gene is present in the *M. abscessus* complex group but absent in *M. chelonae* (*39*). Many strains of *M. abscessus* subsp. *massiliense* have a nonfunctional *erm*(41) gene, and because of this, the rate of clarithromycin susceptibility is higher in *M. abscessus* subsp. *massiliense* than in *M. abscessus* subsp. *abscessus* (*18*). The Clinical and Laboratory Standards Institute recommends testing for inducible macrolide resistance because subspecies of *M. abscessus* complex demonstrate susceptibility to clarithromycin during the first 3–5 days of incubation but demonstrate resistance after an extended duration of incubation (preferably 14 days, according to many experts) (*39*).

Another area of strenuous clinical research involves identifying and developing novel anti–*M. abscessus* complex agents. One such agent, the glycylcycline tigecycline, has been shown to exhibit good in vitro activity against rapidly growing mycobacteria, especially *M. abscessus* complex (*12*,*21*). However, no prospective trial has been conducted to evaluate the efficacy of tigecycline, and a breakpoint for interpreting tigecycline susceptibility has not been established (*32*).

Table 1. Summary of recent data on the resistance of *Mycobacterium abscessus* complex bacteria to different antimicrobial agents*

Study authors (reference), species	No. isolates	Antimicrobial drug, no. resistant isolates/no. tested (%)						
		CLR	DOX	CIP	MXF	FOX	AMK	IPM
Lee et al. (*34*)								
M. abscessus subsp. *abscessus*	202	48/202 (24)	NA	184/202 (91)	167/202 (83)	NA	25/202 (12)	NA
M. abscessus subsp. *massiliense*	199	15/199 (8)	NA	174/199 (87)	149/199 (75)	NA	12/199 (6)	NA
Koh et al. (*18*)								
M. abscessus subsp. *abscessus*	64	3/64 (5)	53/64 (83)	37/64 (58)	30/64 (47)	0/64	3/64 (5)	27/62 (44)
M. abscessus subsp. *massiliense*	79	3/79 (4)	58/79 (73)	48/79 (61)	42/79 (53)	1/79 (1)	6/79 (8)	50/75 (67)
Huang et al. (*35*)								
M. abscessus complex	40	3/40 (8)	37/40 (93)	36/40 (90)	31/40 (78)	27/40 (68)	2/40 (5)	35/40 (88)
Brown-Elliott et al. (*36*)								
M. abscessus complex	37	0% (0/37)	NA	29/37 (78)	29/37 (78)	NA	0/37	7/37 (19)
Broda et al. (*37*)								
M. abscessus complex	58	22/58 (38)	57/58 (98)	55/58 (95)	55/58 (95)	16/58 (28)	10/58 (17)	56/58 (97)
Zhuo et al. (*38*)								
M. abscessus complex	70	10/70 (14)	NA	56/70 (80)	NA	3/70 (4)	0/70	15/70 (21)
Overall								
M. abscessus complex	749	104/749 (13.9)	205/241 (85.1)	619/749 (82.6)	503/679 (74.1)	47/311 (15.1)	58/749 (7.7)	190/342 (55.6)
M. abscessus subsp. *abscessus*	266	51/266 (19.4)	53/64 (83.0)	221/266 (83.1)	197/266 (74.1)	0/64	28/266 (10.5)	27/62 (44.0)
M. abscessus subsp. *massiliense*	278	18/278 (6.5)	58/79 (73.4)	222/278 (79.8)	191/278 (68.7)	1/79 (1.0)	18/278 (6.5)	50/75 (66.7)

*AMK, amikacin; CIP, ciprofloxacin; CLR, clarithromycin; DOX, doxycycline; FOX, cefoxitin; IPM, imipenem; MXF, moxifloxacin; NA, not available.

Treatment

Several problems regarding treatment of *M. abscessus* complex infections in different organs are unsolved. For example, there is a lack of consensus on the optimal antimicrobial agents and combination therapy, optimal treatment duration, and the introduction of novel antimicrobial agents (e.g., tigecycline). Reports describing cases of *M. abscessus* complex infection are limited, except for those describing pulmonary disease and SSTIs. Thus, treatment recommendations must rely on retrospective case series. A summary of treatment recommendations from previous studies is shown in Table 2. The treatment of serious *M. abscessus* complex disease usually involves initial combination antimicrobial therapy with a macrolide (clarithromycin 1,000 mg daily or 500 mg twice daily, or azithromycin 250 mg–500 mg daily) plus intravenous agents for at least 2 weeks to several months followed by oral macrolide–based therapy (*2*). The drugs of choice for initial intravenous administration are amikacin (25 mg/kg 3×/wk) plus cefoxitin (up to 12 g/d given in divided doses) or amikacin (25 mg/kg 3×/wk) plus imipenem (500 mg 2–4×/wk) (*2*). As previously mentioned, the in vitro MICs of tigecycline are low, and the drug should be considered in treatment regimens.

Prevention

M. abscessus complex infection can be acquired in the community or in the hospital setting. In the community setting, water supply systems have been postulated to be the source of human infections (*7*,*40*). Membrane filtration,

Table 2. Summary of recommendations from previous studies for the treatment of *Mycobacterium abscessus* complex infections in humans

Type of disease (reference)	Recommended initial regimen	Recommended treatment duration
Pulmonary disease (*2*)	Macrolide-based therapy in combination with intravenous antimicrobial therapy (preferably cefoxitin and amikacin)	Continue until sputum samples are negative for *M. abscessus* complex for 12 mo
Skin and soft-tissue infection (*2*)	Macrolide in combination with amikacin plus cefoxitin/imipenem plus surgical debridement	Minimum of 4 mo, including a minimum of 2 wk combined with intravenous agents
Central nervous system infection (*21*)	Clarithromycin-based combination therapy (preferably including at least amikacin in the first weeks)	12 mo
Bacteremia (*24*,*25*)	At least 2 active antimicrobial agents (preferably including amikacin) plus removal of catheter and/or surgical debridement of infection foci	4 wk after last positive blood culture result
Ocular infection (*28*)	Topical agents (amikacin, clarithromycin) and/or systemic antimicrobial drugs (oral clarithromycin, intravenous amikacin or cefoxitin) and/or surgical debridement*	6 wk to 6 mo

*The treatment of ocular infections was highly dependent on the infection site. In some sites, ≥1 treatment strategies (i.e., topical or systemic antimicrobial drug treatment or surgery) should be considered.

hyperchlorination, maintenance of constant pressure gradients, and the utilization of particular pipe materials have been suggested as methods for reducing the presence of NTM in water supply systems (*7*,*40*). In the hospital setting, disinfectant failure, contamination of medical devices and water, and indirect transmission between patients are considered to be the source of infections (*19*,*30*). In addition, clinics for cosmetic procedures have become sites of frequent outbreaks of *M. abscessus* complex infections (*19*,*29*). It is unclear whether patients with *M. abscessus* complex disease should be isolated from vulnerable hosts, such as patients with cystic fibrosis.

Conclusions

M. abscessus complex comprises a group of rapidly growing, multidrug-resistant, nontuberculous mycobacteria that are responsible for a wide spectrum of SSTIs and other infections. The complex is differentiated into 3 subspecies: *M. abscessus* subsp. *abscessus, M. abscessus* subsp. *massiliense*, and *M. abscessus* subsp. *bolletii*, which is rarely isolated. The major difference between *M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *abscessus* is that the former does not have an intact *erm*(41) gene and thus does not have inducible macrolide resistance; treatment response may thus be better among patients with infections caused by *M. abscessus* subsp. *massiliense*. *M. abscessus* complex can cause infections involving almost all organs, but the infections generally involve the lungs, skin, and soft tissue. Drugs with the best in vitro activity include clarithromycin, amikacin, cefoxitin, and possibly tigecycline. Treatment regimens vary according to the infection site and usually include macrolide-based combination therapy, including parenteral amikacin plus another parenteral agent (cefoxitin, tigecycline, imipenem, or linezolid), for weeks to months, followed by oral antimicrobial therapy. Evidence of nosocomial transmission and outbreaks of *M. abscessus* complex is increasing; therefore, strenuous infection control measures should be taken to reduce the possibility of hospital-acquired *M. abscessus* complex infections.

Because of the complexity of the molecular techniques needed to differentiate between *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense*, it is difficult for most laboratories to identify the different subspecies. A more rapid and less expensive method for subspecies identification is thus needed for epidemiologic and clinical purposes. In addition, prospective trials comparing different regimens of antimicrobial agents are needed to determine the best treatment options; these studies should include novel agents, such as tigecycline. The effect of implementing isolation protocols for patients with infections due to *M. abscessus* complex (particularly pulmonary disease) should also be evaluated in future studies.

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RESEARCH ARTICLE

Cholelithiasis and Nephrolithiasis in HIV-Positive Patients in the Era of Combination Antiretroviral Therapy

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Abstract

Objectives

This study aimed to describe the epidemiology and risk factors of cholelithiasis and nephrolithiasis among HIV-positive patients in the era of combination antiretroviral therapy.

Methods

We retrospectively reviewed the medical records of HIV-positive patients who underwent routine abdominal sonography for chronic viral hepatitis, fatty liver, or elevated aminotransferases between January 2004 and January 2015. Therapeutic drug monitoring of plasma concentrations of atazanavir was performed and genetic polymorphisms, including UDP-glucuronosyltransferase (UGT) 1A1*28 and multidrug resistance gene 1 (MDR1) G2677T/A, were determined in a subgroup of patients who received ritonavir-boosted or unboosted atazanavir-containing combination antiretroviral therapy. Information on demographics, clinical characteristics, and laboratory testing were collected and analyzed.

Results

During the 11-year study period, 910 patients who underwent routine abdominal sonography were included for analysis. The patients were mostly male (96.9%) with a mean age of 42.2 years and mean body-mass index of 22.9 kg/m^2 and 85.8% being on antiretroviral therapy. The anchor antiretroviral agents included non-nucleoside reverse-transcriptase inhibitors (49.3%), unboosted atazanavir (34.4%), ritonavir-boosted lopinavir (20.4%), and

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ritonavir-boosted atazanavir (5.5%). The overall prevalence of cholelithiasis and nephrolithiasis was 12.5% and 8.2%, respectively. Among 680 antiretroviral-experienced patients with both baseline and follow-up sonography, the crude incidence of cholelithiasis and nephrolithiasis was 4.3% and 3.7%, respectively. In multivariate analysis, the independent factors associated with incident cholelithiasis were exposure to ritonavir-boosted atazanavir for >2 years (adjusted odds ratio [AOR], 6.29; 95% confidence interval [CI], 1.12–35.16) and older age (AOR, 1.04; 95% CI, 1.00–1.09). The positive association between duration of exposure to ritonavir-boosted atazanavir and incident cholelithiasis was also found (AOR, per 1-year exposure, 1.49; 95% CI, 1.05–2.10). The associated factors with incident nephrolithiasis were hyperlipidemia (AOR, 3.97; 95% CI, 1.32–11.93), hepatitis B or C coinfection (AOR, 3.41; 95% CI, 1.09–10.62), and exposure to abacavir (AOR, 12.01; 95% CI, 1.54–93.54). Of 180 patients who underwent therapeutic drug monitoring of plasma atazanavir concentrations and pharmacogenetic investigations, we found that the atazanavir concentrations and UGT 1A1*28 and MDR1 G2677T/A polymorphisms were not statistically significantly associated with incident cholelithiasis and nephrolithiasis.

Conclusions

In HIV-positive patients in the era of combination antiretroviral therapy, a high prevalence of cholelithiasis and nephrolithiasis was observed, and exposure to ritonavir-boosted atazanavir for >2 years was associated with incident cholelithiasis.

Introduction

Both cholelithiasis and nephrolithiasis are widespread conditions constituting a major health burden, affecting an estimated 10–15% and 2–20% of the adult population, respectively [1]. The prevalence and incidence of cholelithiasis and nephrolithiasis vary with geographic locations and have increased over the past decades [2,3]. The increasing rates of cholelithiasis and nephrolithiasis are multifactorial, and several demographic and metabolic factors have been identified as risk factors [1]. In contrast, few studies have investigated the epidemiology of cholelithiasis and nephrolithiasis in people infected with HIV [4,5]. Previous studies have linked protease inhibitors (PIs) to cholelithiasis and nephrolithiasis, for example indinavir, a first-generation PI, which is well known for its crystallization in urine [6]. More recently, ritonavir-boosted atazanavir (atazanavir/ritonavir) has been associated with cholelithiasis and nephrolithiasis [4,7,8]. However, the impact of atazanavir/ritonavir exposure on cholelithiasis and nephrolithiasis remains difficult to estimate since screening methods using sonography were not routinely performed [9].

Modifiable risk factors of cholelithiasis and nephrolithiasis such as offending drugs are worthwhile to identify. In some circumstances, therapeutic drug monitoring (TDM) has been applied to minimize indinavir-related nephrolithiasis [10,11]. While no direct evidence of the association has been established between plasma atazanavir concentrations and cholelithiasis and nephrolithiasis, switch from atazanavir/ritonavir to unboosted atazanavir guided by TDM may reduce atazanavir-related hyperbilirubinemia [12]. On the other hand, UDP-glucuronosyltransferase (UGT) 1A1 and multidrug resistance gene 1 (MDR1) 2677 may also alter plasma atazanavir concentrations, with unknown consequences on the rate of atazanavir-induced cholelithiasis and nephrolithiasis [13,14]. In this study, we aimed to investigate the prevalence and

incidence of cholelithiasis and nephrolithiasis, and to identify their associated factors among HIV-positive Taiwanese patients.

Patients and Methods

Ethics statement

This study was approved by the Research Ethics Committee of National Taiwan University Hospital (registration number, NTUH-201404010RIN). All patients signed written informed consent to provide their clinical and laboratory data for research before recruitment.

Study population and study setting

This retrospective cohort study was conducted at the National Taiwan University Hospital, which is the major designated hospital for HIV care in Taiwan. HIV-positive patients were eligible for recruitment if they were aged 20 years or greater and had undergone routine abdominal sonography for chronic viral hepatitis, fatty liver, or elevated aminotransferases between January 2004 and January 2015. The sonography was performed according to routine clinical practice and not specifically for the study. Both antiretroviral-naive and-experienced patients were included, but patients with uncertain previous antiretroviral regimens and cholecystectomy prior to sonography were excluded.

According to the Taiwanese treatment guidelines for HIV infection, non-nucleoside reverse-transcriptase inhibitor (nNRTI)-containing regimens are the preferred regimens for antiretroviral-naive patients [15]. During the study period, the available PIs included nelfinavir, saquinavir, indinavir, ritonavir-boosted lopinavir, atazanavir, and darunavir.

Data collection

The abdominal sonography was performed by certified physicians, and the hepatobiliary and genitourinary systems were completely imaged on the sonography for detection of gallstones and renal stones. All patients were followed until gallstones or renal stones were detected, they were lost to follow-up, or to 31st January, 2015 when this study terminated, whichever occurred first. Only the sonographic results after the diagnosis of HIV infection were recorded, and the first positive sonographic results or the latest negative sonographic results for gallstones or renal stones were used for analysis in patients having undergone sonography more than once.

A standardized case record form was used to collect clinical information prior to the sonography, which included demographics, body-mass index (BMI), underlying diseases, HIV-related factors such as hepatitis B or C coinfection, history and duration of antiretroviral therapy, baseline and follow-up CD4 lymphocyte counts, plasma HIV RNA loads (PVL), and laboratory data (liver-function tests, lipid profile, estimated glomerular filtration rate [GFR], serum uric acid, urine pH, and urinary crystal). We recorded the history of all prior and current antiretroviral regimens with exposure duration >3 months before abdominal sonography, and a history of exposure to nNRTIs or PIs for >2 years was regarded as a variable in logistic regression according to the previous study [4].

Chronic hepatitis B coinfection was defined as the persistence of hepatitis B virus surface antigen for >6 months, and hepatitis C coinfection was defined by positive anti-HCV antibody. Estimated GFR was calculated with the use of the Modification of Diet in Renal Diseases (MDRD) Study equation. The baseline values of CD4 lymphocyte count and PVL were obtained at the diagnosis of HIV. Sequential laboratory parameters were updated within 6 months before abdominal sonography.

Laboratory investigations

CD4 lymphocyte count and plasma HIV RNA load. CD4 lymphocyte count was determined using flow cytometry (BD FACS Calibur, Becton Dickinson and Coulter Epics XL, Beckman Coulter, CA, USA). PVL was quantified using the Cobas AmpliPrep/Cobas TaqMan HIV-I test (version 2.0, Roche Molecular Systems, Inc.) with a lower detection limit of 20 copies/mL since June 2012.

Determination of plasma atazanavir concentrations. To optimize antiretroviral therapy, TDM of the two most commonly used antiretroviral agents, efavirenz and atazanavir, has been available at this hospital since 2009 [16]. In this study, the patients who underwent TDM of plasma atazanavir concentrations and determinations of genetic polymorphisms and had undergone sonography were included to investigate the correlation between stone formation and plasma atazanavir concentrations. We collected the closest data that antedated the date of abdominal sonography. The recommended trough plasma concentration (C_{trough}) of atazanavir ranges between 0.15 and 0.85 mg/L, which is associated with virological suppression and less unconjugated hyperbilirubinemia [17]. Either mid-dosing interval (C_{12}) or trough (C_{24}) concentration was determined using high-performance liquid chromatography (HPLC) based on a validated method by Ramachandran et al. with minor modifications [18]. The detailed methods for determination of plasma atazanavir concentrations are available in the supplement (**S1 Text**).

Genetic polymorphisms. Two genetic polymorphisms of UGT 1A1 and MDR1 2677 were determined according to previous studies with minor modifications [19,20]. Genomic DNA was extracted from peripheral blood mononuclear cell with use of the Wizard Genomic DNA purification kit (Promega, WI, USA). The TATA box of the UGT1A1 promoter and genotypes at the MDR1 locus were determined by polymerase chain reaction (PCR) amplification and further sequenced or restriction fragment-length polymorphism (RFLP) analysis, respectively. The other detailed methods for determination of genetic polymorphisms are available in the supplement (**S1 Text**).

Investigations of prevalence and incidence of and factors associated with cholelithiasis and nephrolithiasis

This study comprised separate analyses (**Fig 1**). Analysis 1 included all eligible HIV-positive patients who had undergone routine abdominal sonography to estimate the prevalence of cholelithiasis and nephrolithiasis and their associated factors. Analysis 2 included only the antiretroviral-experienced patients who had undergone sonography before and after initiating antiretroviral therapy to determine the crude incidence of cholelithiasis and nephrolithiasis and their associated factors. Patients in analysis 2 were classified as antiretroviral-experienced patients who did not develop cholelithiasis and/or nephrolithiasis and antiretroviral-experienced patients who developed cholelithiasis and/or nephrolithiasis after therapy. Analysis 3 included only the subset of patients on atazanavir for more than 3 months with TDM and genetic polymorphism data to determine the effect of plasma atazanavir concentrations and genetic polymorphisms on the occurrence of incident cholelithiasis and nephrolithiasis.

Statistical analysis

We used descriptive statistics to evaluate distributions of patients' demographics and clinical characteristics. Categorical variables were compared with a chi-square test or Fisher's exact test if the expected values were <10. Continuous variables were described as mean ± standard deviation (SD), and were compared using the Student's *t*-test, or were described as the median and range, and were compared with the Wilcoxon rank-sum test if their distributions were not normal. All

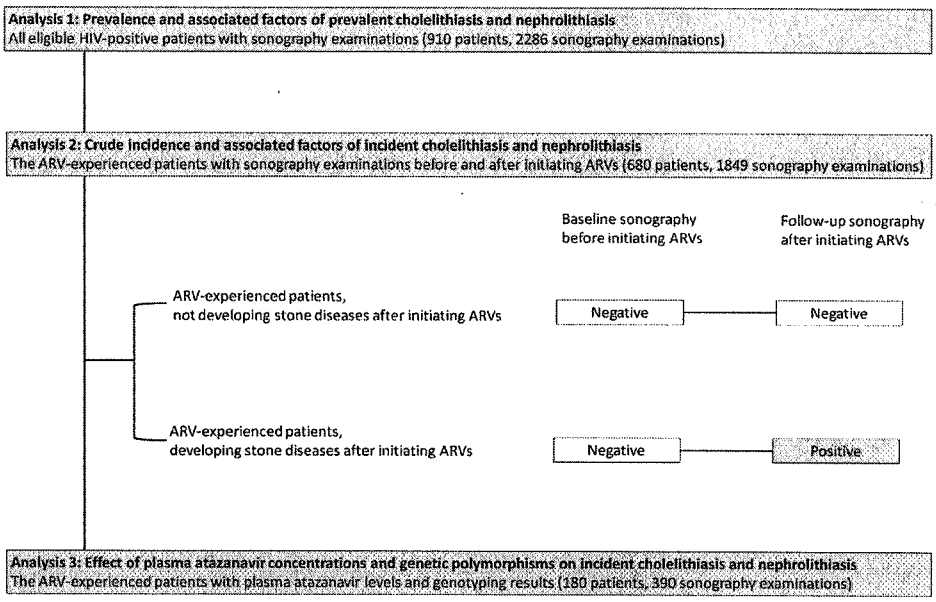


Fig 1. Flow diagram of patient selection for three analyses. Abbreviation: ARV, antiretroviral.

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tests were two-tailed and $P < 0.05$ was considered statistically significant. The crude incidence of stone disease was the rate at which new cases with cholelithiasis or nephrolithiasis occurred during the study period. Associated factors with prevalent or incident cholelithiasis and nephrolithiasis were identified using multivariate logistic regression model. Variables considered for entry into multivariate logistic regression model included variables with P values <0.10 in univariate analysis. 95% confidence intervals (CIs) of odds ratios (ORs) were computed to estimate the effects of each variable on cholelithiasis and nephrolithiasis. Statistical analyses were performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA).

Results

Analysis one: the prevalence and factors associated with prevalent cholelithiasis and nephrolithiasis

During the 11-year study period, we included 910 HIV-positive Taiwanese patients who had undergone abdominal sonography for chronic hepatitis B or C (464/910, 51.0%), fatty liver (343/910, 37.7%), or elevated aminotransferases (268/910, 29.5%). The total number of sonography performed among these patients was 2286, and the median interval of follow-up sonography was 329 days (interquartile range [IQR], 189–490 days). The demographics and clinical characteristics of these 910 patients are summarized in **Table 1**. Most patients were male (96.9%) with a mean age of 42.2 years and mean BMI of 22.9 kg/m^2. Nearly half (51.0%) of the patients had had a history of chronic hepatitis B or C coinfection, for which serial follow-up abdominal sonography was performed. The major underlying diseases were hyperlipidemia (20.3%), followed by chronic hepatitis (12.6%) and hypertension (10.9%). The percentage of patients who were taking antiretroviral therapy was 85.8% (781/910), and the anchor antiretroviral agents combined with 2 NRTIs included nNRTIs (49.3%), unboosted atazanavir (34.4%), lopinavir/ritonavir (20.4%), and atazanavir/ritonavir (5.5%).

Table 1. The demographics and clinical characteristics of 910 HIV-positive patients who had undergone abdominal sonography.

Variable	All patients (N = 910)
Demographics	
Age, mean ± SD, years	42.2 ± 10.6
Male sex, n (%)	882 (96.9)
Body-mass index, mean ± SD, kg/m^2	22.9 ± 3.5
Underlying diseases, n (%)	
Hyperlipidemia	185 (20.3)
Chronic hepatitis[a]	115 (12.6)
Hypertension	99 (10.9)
Diabetes mellitus	56 (6.2)
Liver cirrhosis	26 (2.9)
Chronic kidney disease	20 (2.2)
HIV-related factors	
Homosexual male, n (%)	711 (78.1)
Hepatitis B or C coinfection, n (%)	464 (51.0)
Duration of HIV infection, mean ± SD, years	6.4 ± 5.3
Duration of antiretroviral therapy, mean ± SD, years	4.9 ± 4.5
History of antiretroviral therapy, n (%)	
Antiretroviral-experienced	781 (85.8)
Zidovudine	291 (32.0)
Abacavir	422 (46.4)
Tenofovir	442 (48.6)
Other NRTIs[b]	149 (16.4)
NNRTI	449 (49.3)
Unboosted atazanavir	313 (34.4)
Atazanavir/ritonavir	50 (5.5)
Lopinavir/ritonavir	186 (20.4)
Darunavir/ritonavir	29 (3.2)
Indinavir/ritonavir	38 (4.2)
Baseline laboratory investigations[c]	
Baseline PVL, median (range), $\log_{10}$ copies/mL	5.08 (1.94–7.23)
Baseline CD4 count, median (range), cells/μL	151.0 (0–1265.0)
Follow-up laboratory investigations[d]	
Follow-up PVL, median (range), $\log_{10}$ copies/mL	UD (UD-7.00)
Follow-up CD4 count, median (range), cells/μL	533.0 (1.0–2091.0)

Abbreviations: NNRTI, non-nucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PVL, plasma HIV RNA load; SD, standard deviation; UD, undetectable.

[a]Chronic hepatitis was defined as persistent elevation in serum aminotransferases for 6 months or longer.

[b]Other NRTIs included stavudine, didanosine, and zalcitabine.

[c]Baseline laboratory investigations were the laboratory data obtained at the diagnosis of HIV.

[d]Follow-up laboratory investigations were the laboratory data obtained within 6 months before abdominal sonography.

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The overall prevalence of cholelithiasis and/or nephrolithiasis was 18.8% (171/910), which included 12.5% (114/910) for cholelithiasis and 8.2% (75/910) for nephrolithiasis. Only 19 patients (19/171, 11.1%) had subsequent complications (i.e. cholecystitis and hydronephrosis) and 16 patients received further interventions to relieve the symptoms. In antiretroviral-naïve and antiretroviral-experienced patients, the prevalence of cholelithiasis and/or nephrolithiasis

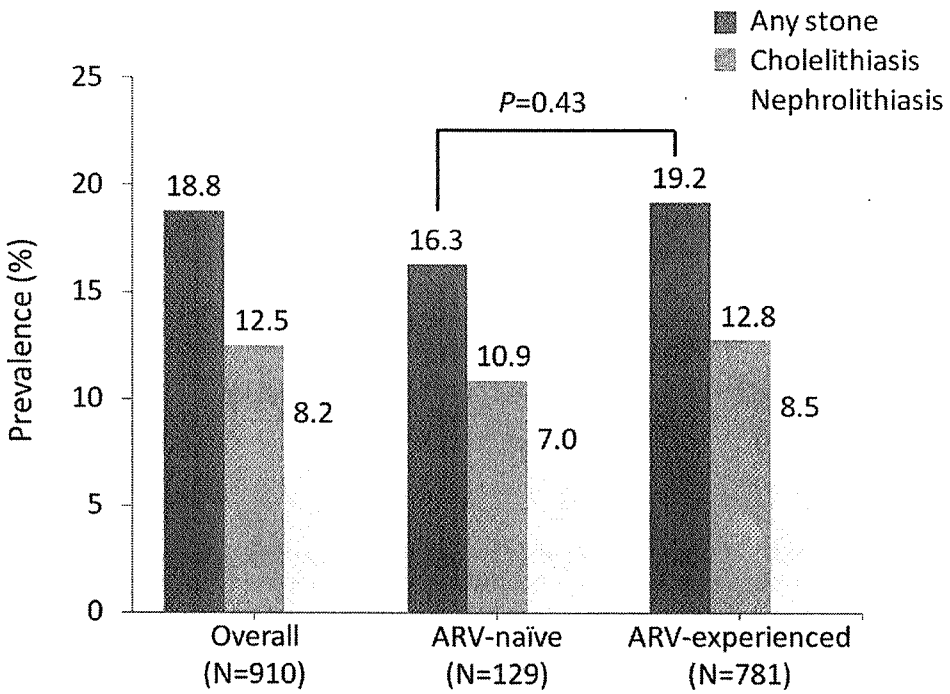


Fig 2. The prevalence of cholelithiasis and/or nephrolithiasis in 910 HIV-positive patients who had undergone abdominal sonography.
Abbreviation: ARV, antiretroviral.

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was 16.3% (21/129) and 19.2% (150/781), respectively ($P = 0.43$) (Fig 2). The prevalence of cholelithiasis and/or nephrolithiasis in patients with exposure to atazanavir or atazanavir/ritonavir, PIs other than atazanavir, and nNRTIs was 22.0%, 20.8%, and 18.9%, respectively (all $P>0.05$ compared with antiretroviral-naïve patients). In multivariate analysis, the independent factors associated with prevalent cholelithiasis were exposure to atazanavir/ritonavir for >2 years (adjusted OR [AOR], 4.70; 95% CI, 1.34–16.54), older age (AOR, per 1-year increase, 1.04; 95% CI, 1.01–1.06), duration of antiretroviral therapy (AOR, per 1-year increase, 0.91; 95% CI, 0.84–0.99), and elevated serum total bilirubin (AOR, per 1-mg/dL increase, 1.29; 95% CI, 1.05–1.57) (S1 Table). For nephrolithiasis, the associated factors were increasing age (AOR, per 1-year increase, 1.03; 95% CI, 1.00–1.06), decreased estimated GFR (AOR, per 1-ml/min/1.73m^2 decrease, 1.02; 95% CI, 1.01–1.03), and elevated serum total cholesterol (AOR, per 1-mg/dL increase, 1.01; 95% CI, 1.01–1.02) (S1 Table).

Analysis two: crude incidence and factors associated with incident cholelithiasis and nephrolithiasis

A total of 680 antiretroviral-experienced patients who had undergone sonography before and after initiating antiretroviral therapy were included to estimate the crude incidences of cholelithiasis and nephrolithiasis. The total number of sonography performed among these patients was 1849, and the median interval of follow-up sonography was 332 days (IQR, 189–492 days). The indications of sonography were hepatitis B or C (346/680, 50.9%), fatty liver (240/680, 35.3%), or elevated aminotransferases (142/680, 20.9%). The demographics and clinical characteristics of patients with or without incident cholelithiasis and nephrolithiasis are shown in Table 2. The cumulative crude incidence of developing cholelithiasis and/or nephrolithiasis

Table 2. The demographics and clinical characteristics of 680 antiretroviral-experienced patients with or without incident cholelithiasis and nephrolithiasis.

Variable	Cohort 1[a]			Cohort 2[a]		
	Cholelithiasis (N = 29)	No cholelithiasis (N = 651)	*P*	Nephrolithiasis (N = 25)	No nephrolithiasis (N = 655)	*P*
Demographics						
Age, mean ± SD, years	47.4 ± 9.8	42.1 ± 10.7	0.002	48.6 ± 12.4	42.1 ± 10.5	0.001
Male sex, n (%)	27 (93.1)	635 (97.5)	0.18	23 (92.0)	639 (97.6)	0.14
Body-mass index, mean ± SD, kg/m^2	23.9 ± 3.4	22.8 ± 3.4	0.17	22.1 ± 3.4	22.9 ± 3.3	0.13
Underlying diseases, n (%)						
Hyperlipidemia	7 (24.1)	133 (20.4)	0.64	9 (36.0)	131 (20.0)	0.07
Chronic hepatitis[b]	7 (24.1)	71 (10.9)	0.04	5 (20.0)	73 (11.1)	0.19
Hypertension	4 (13.8)	60 (9.2)	0.34	6 (24.0)	58 (8.9)	0.02
Diabetes mellitus	3 (10.3)	31 (4.8)	0.17	1 (4.0)	33 (5.0)	0.99
Liver cirrhosis	1 (3.4)	17 (2.6)	0.55	1 (4.0)	17 (2.6)	0.50
Chronic kidney disease	1 (3.4)	13 (2.0)	0.46	2 (8.0)	12 (1.8)	0.09
HIV-related factors						
Homosexual male, n (%)	23 (79.3)	523 (80.3)	0.82	17 (68.0)	529 (80.8)	0.13
Hepatitis B or C coinfection, n (%)	19 (65.5)	327 (50.2)	0.11	18 (72.0)	328 (50.1)	0.04
Duration of HIV infection, mean ± SD, years	8.3 ± 4.4	7.0 ± 5.2	0.07	9.5 ± 5.7	7.0 ± 5.1	0.02
Duration of antiretroviral therapy, mean ± SD, years	6.8 ± 3.8	5.6 ± 4.2	0.05	7.6 ± 5.0	5.6 ± 4.1	0.04
History of antiretroviral therapy, n (%)						
Zidovudine	11 (37.9)	234 (35.9)	0.83	11 (44.0)	234 (35.7)	0.40
Abacavir	20 (69.0)	345 (53.0)	0.13	19 (76.0)	346 (52.8)	0.03
Tenofovir	14 (48.3)	377 (57.9)	0.30	11 (44.0)	380 (58.0)	0.16
NNRTI	17 (58.6)	377 (57.9)	0.94	16 (64.0)	378 (57.7)	0.68
Duration of exposure, mean ± SD, years	2.5 ± 3.2	2.5 ± 3.4	0.88	4.3 ± 4.5	2.4 ± 3.4	0.06
> 2 years, n (%)	11 (37.9)	242 (37.2)	0.93	14 (56.0)	239 (36.5)	0.05
Unboosted atazanavir	16 (55.2)	250 (38.4)	0.07	11 (44.0)	255 (38.9)	0.61
Duration of exposure, mean ± SD, years	1.9 ± 2.3	1.3 ± 2.3	0.06	1.2 ± 1.8	1.3 ± 2.3	0.79
> 2 years, n (%)	12 (41.4)	167 (25.7)	0.06	6 (24.0)	173 (26.4)	0.99
Atazanavir/ritonavir	4 (13.8)	41 (6.3)	0.12	1 (4.0)	44 (6.7)	0.99
Duration of exposure, mean ± SD, years	0.5 ± 1.4	0.1 ± 0.6	0.09	0.04 ± 0.2	0.1 ± 0.7	0.58
> 2 years, n (%)	3 (10.3)	10 (1.5)	0.02	0 (0)	13 (2.0)	0.99
Lopinavir/ritonavir	7 (24.1)	153 (23.5)	0.99	5 (20.0)	155 (23.7)	0.81
Duration of exposure, mean ± SD, years	1.3 ± 2.8	0.9 ± 2.1	0.75	1.2 ± 2.9	0.9 ± 2.1	0.85
> 2 years, n (%)	6 (20.7)	110 (16.9)	0.61	5 (20.0)	111 (16.9)	0.60
Darunavir/ritonavir	1 (3.4)	25 (3.8)	0.99	0 (0)	26 (4.0)	0.62
Duration of exposure, mean ± SD, years	± 0.3	0.1 ± 0.3	0.92	0	0.1 ± 0.3	0.31
> 2 years, n (%)	0 (0)	8 (1.2)	0.99	0 (0)	8 (1.2)	0.99
Indinavir/ritonavir	2 (6.9)	26 (4.0)	0.34	1 (4.0)	27 (4.1)	0.99
Duration of exposure, mean ± SD, years	0.3 ± 1.3	0.2 ± 1.2	0.45	0.2 ± 1.1	0.2 ± 1.2	0.97

(*Continued*)

Table 2. (*Continued*)

Variable	Cohort 1[a]			Cohort 2[a]		
	Cholelithiasis (N = 29)	No cholelithiasis (N = 651)	*P*	Nephrolithiasis (N = 25)	No nephrolithiasis (N = 655)	*P*
> 2 years, n (%)	1 (3.4)	22 (3.4)	0.99	1 (4.0)	22 (3.4)	0.58
Baseline laboratory investigations[c]						
Baseline PVL, median (range), log_{10} copies/mL	5.42 (4.14–7.00)	5.05 (1.94–7.00)	0.05	5.35 (3.91–5.96)	5.08 (1.94–7.00)	0.21
Baseline CD4 count, median (range), cells/μL	67.0 (1–591.0)	157.0 (0–1265.0)	0.22	84.0 (0–448.0)	155.0 (0–1265.0)	0.10
Follow-up laboratory investigations[d]						
Follow-up PVL, median (range), log_{10} copies/mL	UD (UD-4.91)	UD (UD-5.27)	0.07	UD (UD-2.96)	UD (UD-5.27)	0.41
Follow-up CD4 count, median (range), cells/μL	502.0 (4.0–1316.0)	565.0 (8.0–1965.0)	0.32	458.5 (76.0–1012.0)	565.0 (4.0–1965.0)	0.27
Estimated GFR, mean ± SD, mL/min/ $1.73m^2$	92.5 ± 19.5	99.1 ± 21.6	0.12	93.0 ± 32.5	99.1 ± 21.0	0.33
Serum total bilirubin, mean ± SD, mg/ dL	2.2 ± 3.9	1.2 ± 1.0	0.25	1.4 ± 1.7	1.2 ± 1.2	0.57
Serum ALT, mean ± SD, U/L	49.1 ± 86.3	34.5 ± 37.9	0.25	31.3 ± 24.7	35.3 ± 41.6	0.93
Serum total cholesterol, mean ± SD, mg/dL	173.7 ± 35.4	171.6 ± 35.1	0.72	189.3 ± 40.5	171.1 ± 34.7	0.02
Serum triglyceride, mean ± SD, mg/ dL	163.0 ± 104.6	155.5 ± 129.6	0.37	170.0 ± 82.8	155.3 ± 130.0	0.10
Serum uric acid, mean ± SD, mg/dL	NA	NA	NA	4.9 ± 0.0	6.2 ± 2.0	0.39
Urine pH, mean ± SD, unit	NA	NA	NA	6.2 ± 0.6	6.0 ± 0.6	0.10
Urinary crystal, n (%)	NA	NA	NA	0 (0)	15 (2.3)	0.99

Abbreviations: ALT, alanine aminotransferase; GFR, glomerular filtration rate; NNRTI, non-nucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PVL, plasma HIV RNA load; SD, standard deviation; UD, undetectable.

[a]Cohort 1 was the cohort for the comparison between patients with and those without incident cholelithiasis, and cohort 2 was the cohort for the comparison between patients with and those without incident nephrolithiasis.

[b]Chronic hepatitis was defined as persistent elevation in serum aminotransferases for 6 months or longer.

[c]Baseline laboratory investigations were the laboratory data obtained at the diagnosis of HIV.

[d]Follow-up laboratory investigations were the laboratory data obtained within 6 months before abdominal sonography.

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was 7.2% (4 9/680), which included 4.3% (29/680) for cholelithiasis and 3.7% (25/680) for nephrolithiasis after initiating antiretroviral therapy. The crude incidence in patients receiving regimens containing nNRTI, unboosted/boosted atazanavir, and PIs other than atazanavir was 7.6%, 8.8%, and 6.8%, respectively. The crude incidences were not statistically different among the patients receiving different antiretroviral regimens ($P>0.05$).

In univariate analysis, the factors associated with incident cholelithiasis were advanced age, chronic hepatitis, exposure to atazanavir/ritonavir for >2 years, higher baseline or follow-up PVL, and elevated serum total bilirubin (**S2 Table**). For incident nephrolithiasis, the associated factors were advanced age, hypertension, hepatitis B or C coinfection, longer duration of HIV infection or antiretroviral therapy, exposure to abacavir, and elevated serum total cholesterol (all $P<0.05$) (**S2 Table**). In multivariate analysis, the independent factors associated with incident cholelithiasis included exposure to atazanavir/ritonavir for >2 years (AOR, 6.29; 95% CI, 1.12–35.16; $P = 0.04$) and advanced age (AOR, per 1-year increase, 1.04; 95% CI, 1.00–1.09; $P = 0.047$) (**Table 3**). If we considered atazanavir/ritonavir exposure as a continuous variable, a

Table 3. Multivariate logistic analysis to identify the factors associated with incident cholelithiasis in 680 antiretroviral-experienced patients.

Variable[a]	Cholelithiasis		
	OR	95% CI	*P*
Age, per 1-year increase	1.04	1.00–1.09	0.047
Chronic hepatitis	0.74	0.07–7.67	0.80
Unboosted atazanavir, > 2 years	1.42	0.44–4.53	0.56
Atazanavir/ritonavir, > 2 years	6.29	1.12–35.16	0.04
Baseline PVL, per 1-$\log_{10}$ copies/mL increase	1.97	0.92–4.21	0.08
Follow-up PVL, per 1-$\log_{10}$ copies/mL increase	1.22	0.76–1.96	0.40
Serum total bilirubin, per 1-mg/dL increase	1.19	0.78–1.82	0.42
Serum ALT, per 1-U/L increase	1.00	0.96–1.02	0.43

Abbreviations: ALT, alanine aminotransferase; CI, confidence interval; OR, odds ratio; PVL, plasma HIV RNA load.

[a]Variables considered for entry into multivariate logistic regression model included variables with *P* values <0.10 in univariate analysis.

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positive association between the cumulative exposure and incident cholelithiasis was also found (AOR, per 1-year exposure, 1.49; 95% CI, 1.05–2.10; $P = 0.02$). For nephrolithiasis, the associated factors were hyperlipidemia (AOR, 3.97; 95% CI, 1.32–11.93; $P = 0.01$), hepatitis B or C coinfection (AOR, 3.41; 95% CI, 1.09–10.62; $P = 0.04$), and exposure to abacavir (AOR, 12.01; 95% CI, 1.54–93.54; $P = 0.02$) (**Table 4**). There were no cases of nephrolithiasis among those exposed to atazanavir/ritonavir for >2 years or those exposed to darunavir/ritonavir. The cumulative exposure of atazanavir/ritonavir was also not statistically significantly correlated with incident nephrolithiasis.

Table 4. Multivariate logistic analysis to identify the factors associated with incident nephrolithiasis in 680 antiretroviral-experienced patients.

Variable[a]	Nephrolithiasis		
	OR	95% CI	*P*
Age, per 1-year increase	1.03	0.98–1.08	0.21
Hyperlipidemia	3.97	1.32–11.93	0.01
Hypertension	0.48	0.07–3.43	0.47
Chronic kidney disease	2.99	0.28–32.25	0.37
Hepatitis B or C coinfection	3.41	1.09–10.62	0.04
Duration of HIV infection, per 1-year increase	1.08	0.96–1.23	0.21
Duration of antiretroviral therapy, per 1-year increase	0.91	0.78–1.07	0.25
Use of abacavir	12.01	1.54–93.54	0.02
NNRTI exposure > 2 years	1.14	0.34–3.78	0.83
Serum total cholesterol, per 1-mg/dL increase	1.00	0.99–1.02	0.62
Urine pH, per 1-unit increase	2.31	0.98–5.45	0.06

Abbreviations: CI, confidence interval; NNRTI, non-nucleoside reverse-transcriptase inhibitor; OR, odds ratio.

[a]Variables considered for entry into multivariate logistic regression model included variables with *P* values <0.10 in univariate analysis.

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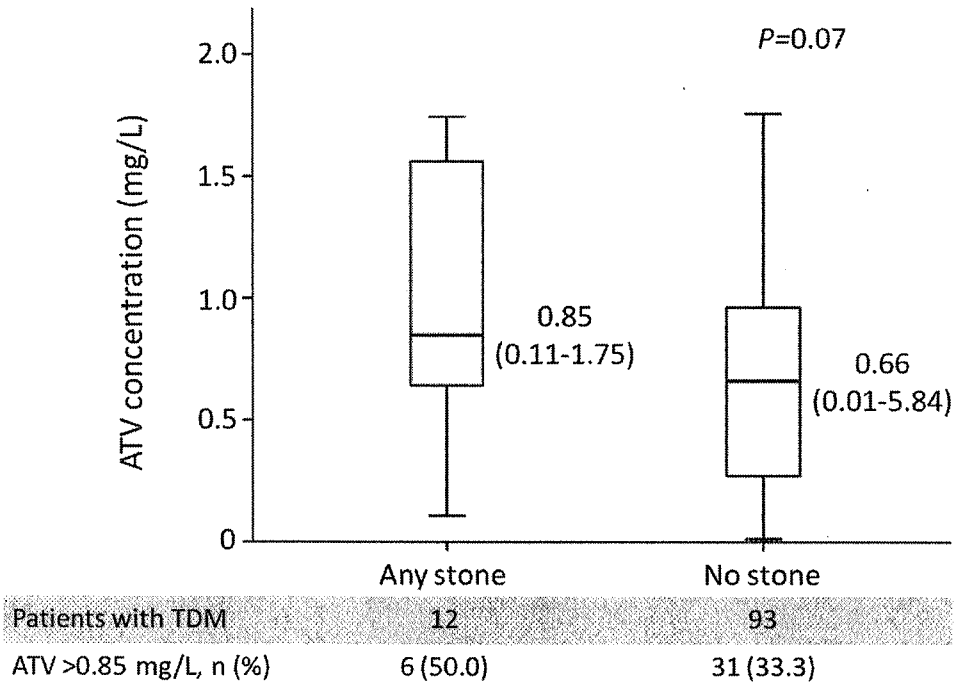


Fig 3. Plasma atazanavir concentrations (C_{12}) of patients with (N = 12) and without (N = 93) incident cholelithiasis and/or nephrolithiasis. **Abbreviations:** ATV, atazanavir; TDM, therapeutic drug monitoring.

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Analysis three: effects of plasma atazanavir concentrations and genetic polymorphisms on incident cholelithiasis and nephrolithiasis

A total of 180 patients underwent TDM of plasma atazanavir concentrations, including 16 patients who developed cholelithiasis and/or nephrolithiasis and 164 patients who did not. The total number of sonography performed among these patients was 390, and the median interval of follow-up sonography was 375 days (IQR, 189–734 days). The indications of sonography were hepatitis B or C (65/180, 36.1%), fatty liver (81/180, 45.0%), or elevated aminotransferases (36/180, 20.0%). The atazanavir C_{12} and C_{24} were compared separately. Fig 3 shows the comparison of atazanavir C_{12} between 12 patients with and 93 patients without incident cholelithiasis and/or nephrolithiasis. The atazanavir C_{12} was higher (median, 0.85 mg/L; range, 0.11–1.75) in patients with incident stones compared with patients without stones (median, 0.66 mg/L; range, 0.01–5.84), but the difference was of borderline significance ($P = 0.07$). The percentage of patients with atazanavir C_{12} above 0.85 mg/L, the upper limit of the therapeutic window, was 50.0% (6/12) and 33.3% (31/93) in patients with and without incident stones, respectively ($P = 0.34$). Only 4 patients with incident stones had atazanavir C_{24} levels, which were lower than that in patients without stones, but the difference was not statistically significant ($P = 0.25$).

The genotyping results of UGT1A1*28 and MDR1 2677, which may affect atazanavir metabolism and lead to hyperbilirubinemia, are shown in Table 5. The frequency of the UGT1A1*28 allele in 16 patients with and 161 patients without incident stones was 12.5% and 19.9%, respectively ($P = 0.74$) and that of the MDR1 G2677T/A in 7 patients with and 86 patients without incident stones was 71.4% and 70.9%, respectively ($P = 0.99$).

Table 5. Comparisons of genotype frequencies for UGT1A1 and MDR1 2677 between patients with and without incident cholelithiasis and/or nephrolithiasis.

Genotype	Any stonen/N (%)[a]	No stone n/N (%)[a]	*P*
UGT1A1*28			
TA6/TA6	14/16 (87.5)	129/161 (80.1)	0.74
TA6/TA7	2/16 (12.5)	32/161 (19.9)	
MDR1 2677			
G/G	2/7 (28.6)	25/86 (29.1)	0.99
G/T	2/7 (28.6)	29/86 (33.7)	
T/T	1/7 (14.3)	12/86 (14.0)	
T/A	1/7 (14.3)	6/86 (7.0)	
G/A	1/7 (14.3)	13/86 (15.1)	
A/A	0/7 (0)	1/86 (1.2)	
MDR1 2677 (T or A)	5/7 (71.4)	61/86 (70.9)	0.99

Abbreviations: MDR1, multidrug resistance gene 1; UGT, UDP-glucuronosyltransferase.

[a]N was the total number of patients with determinations of UGT1A1*28 or MDR1 2677 polymorphisms, and n was the number of patients with specific genotypes.

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Discussion

This study demonstrates a high prevalence of cholelithiasis and nephrolithiasis in the HIV-positive Taiwanese population receiving routine abdominal sonography for chronic viral hepatitis and other indications. Exposure to atazanavir/ritonavir for over 2 years is associated with a 6.29-fold increase in the risk for incident cholelithiasis. However, plasma atazanavir concentrations and genetic polymorphisms related to metabolism of atazanavir are not identified as associated factors, perhaps due to insufficient sample size. To the best of our knowledge, this study is the first analysis that attempts to elucidate the correlation between plasma atazanavir concentrations and stone formation using routine follow-up sonography.

The prevalence and incidence of cholelithiasis and nephrolithiasis are enormously affected by geography and ethnicity, and are relatively lower in Asian countries compared with Western countries [2,3]. The prevalence and incidence of gallstones have been reported to be 4.6–10.7% and 6.32 per 1000 person-years, respectively, in the general Asian population [21,22], with the prevalence and incidence of renal stones being 7.4% in the Taiwanese population, and 1.14 per 1000 person-years in the Japanese population [23,24]. In the HIV-positive population, recent studies conducted in Japan reported higher rates of cholelithiasis (9.8%) and nephrolithiasis (7.1%) by means of clinical diagnosis and imaging performed for various indications [4,7]. To avoid overestimation, we enrolled patients who underwent routine abdominal sonography since sonography has been accepted as the initial imaging modality of choice for the detection of gallstones and renal stones [2,25]. Our findings, similar to those previously reported by Japanese investigators [4,7], also showed a high prevalence of cholelithiasis (12.5%) and nephrolithiasis (8.2%), which was previously attributed to antiretrovirals, especially exposure to PIs [4,6,7,8]. In our study, the high rates of cholelithiasis and nephrolithiasis were found both in antiretroviral-naïve and antiretroviral-experienced patients without statistically significant difference, suggesting that there may be other contributing factors in addition to antiretrovirals in the HIV-positive population. Because the majority of our patients had chronic hepatitis B or C infection (51.0%), the high rates in antiretroviral-naïve patients may result from the impact of hepatitis C coinfection, which has been recognized as a risk factor for cholelithiasis [26].

Whether HIV infection itself may contribute to cholelithiasis and nephrolithiasis warrants further investigations.

Some important risk factors for cholelithiasis and nephrolithiasis have been identified previously [2,27], such as older age, female sex, metabolic syndrome, liver diseases, specific diet and drugs for cholelithiasis and male sex, metabolic syndrome, reduced urinary volume and urine pH, hyperuricosuria, and specific diet and drugs for nephrolithiasis. Among the HIV-infected patients, previously identified risk factors were hepatitis B or C coinfection and exposure to PIs, including indinavir and atazanavir [6,28,29]. Atazanavir-induced cholelithiasis and nephrolithiasis were observed in patients with cumulative exposure to atazanavir/ritonavir for >2 years [4,30–32]. These findings were based on the clinical practice that most patients received atazanavir/ritonavir as their PI-based regimens. In our study, a substantial proportion of the patients received unboosted atazanavir (34.4%) instead of atazanavir/ritonavir (5.5%). The independent factors associated with incident cholelithiasis were exposure to atazanavir/ritonavir for >2 years and older age. Our findings indicate that patients with cumulative exposure to atazanavir/ritonavir, rather than unboosted atazanavir, had a 6.29-fold increase in the risk for incident cholelithiasis. In contrast, our study failed to correlate nephrolithiasis with cumulative exposure to atazanavir/ritonavir. A recent retrospective study using insurance databases also showed no evidence of an increased risk of nephrolithiasis among patients on atazanavir compared with other PIs, but a positive association was observed when atazanavir was compared with PI-free regimens [33]. While no previous studies have ever identified an association between exposure to abacavir and incident nephrolithiasis, the positive association found in our study suggests the direct effect of abacavir or the fact that patients with decreased estimated GFR were more likely to receive abacavir. More studies are needed to confirm our findings.

Two mechanisms are hypothesized for atazanavir-induced cholelithiasis and nephrolithiasis, including precipitation of atazanavir in the less acidic bile and urine and atazanavir-related hyperbilirubinemia facilitating the formation of gallstones [29,34]. To elucidate the effect of atazanavir exposure, plasma atazanavir concentrations and genetic polymorphisms altering the metabolism and transportation of atazanavir need to be examined. To date, only one recent study addressed the relationship between genetic polymorphisms of UGT1A-3'-UTR and atazanavir-induced nephrolithiasis [13]. We examined two well-known genetic polymorphisms of UGT1A1*28 and MDR1 2677 related to atazanavir-induced hyperbilirubinemia [14], but failed to demonstrate statistically significant correlation between the genetic polymorphisms and incident cholelithiasis and nephrolithiasis. In terms of the plasma atazanavir concentrations, atazanavir C_{12} was numerically higher in patients with incident cholelithiasis and nephrolithiasis without reaching statistical significance, which is likely caused by the small sample size in our study. The minimum sample size in each group to detect a difference in atazanavir levels with a power of 80% at 95% confidence level estimated by STPLAN 4.5 would be 100. While our results should be interpreted with caution, our findings suggest that cumulative atazanavir exposure has more impact than plasma drug levels on stone formation.

Our study has several limitations. First, the indications for sonography in our study may cause overestimation of the prevalence and incidence of stone formation. On the other hand, the sensitivity of abdominal sonography for cholelithiasis and nephrolithiasis has been reported to be 97% and 54%, respectively, meaning that some stones especially ureteral stones may be missed by sonography [25, 35]. Our results may not be generalizable to those patients who did not meet the indications for sonography. Second, the follow-up of abdominal sonography was not performed at regular intervals, which made it difficult for us to estimate the incidence rates in person-years. In addition, patients without baseline abdominal sonography prior to initiation of antiretroviral therapy might cause overestimates of prevalence in treatment-

experienced patients. In this study, we used crude incidence instead and excluded patients without baseline sonography from the analysis for identifying associated factors. Third, 180 patients with atazanavir exposure had determinations of plasma atazanavir concentrations and genetic polymorphisms and only 16 patients had cholelithiasis and/or nephrolithiasis. The small sample size may preclude us from identifying correlations between plasma atazanavir concentrations, genetic polymorphisms, and cholelithiasis and nephrolithiasis. Fourth, the information on the previous history of cholelithiasis and nephrolithiasis, diet and other medications except antiretroviral therapy that could contribute to cholelithiasis and nephrolithiasis may not have been recorded in the medical records, and the stone composition was not known. Therefore, we were not able to rule out other contributing factors. Lastly, our study included only Taiwanese and the results may not be generalizable to HIV-positive patients of other ethnicities in other geographic locations.

In conclusion, in HIV-positive Taiwanese patients who had undergone routine abdominal sonography for chronic viral hepatitis and other indications, a high prevalence of cholelithiasis and nephrolithiasis was observed. The cumulative exposure to atazanavir/ritonavir for >2 years was associated with incident cholelithiasis. With the limitation of insufficient sample size, we failed to demonstrate statistically significant associations between plasma atazanavir concentrations, genetic polymorphisms altering atazanavir metabolism and incident cholelithiasis and nephrolithiasis.

Supporting Information

S1 Table. Logistic analysis to estimate the factors associated with prevalent cholelithiasis and nephrolithiasis in 910 HIV-positive patients who had undergone abdominal sonography.
(DOCX)

S2 Table. Univariate logistic analysis to estimate the factors associated with incident cholelithiasis and nephrolithiasis in 680 antiretroviral-experienced patients.
(DOCX)

S1 Text. The detailed methods for determination of plasma atazanavir concentrations and genetic polymorphisms.
(DOCX)

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Author Contributions

Conceived and designed the experiments: KYL SHL HPW CCH. Performed the experiments: KYL SHL WCL SWL SYC MRW HPW CCH. Analyzed the data: KYL SHL WCL SWL SYC MRW HPW CCH AC. Contributed reagents/materials/analysis tools: KYL SHL WCL SWL SYC MST CHK MRW HPW CCH SCC. Wrote the paper: KYL SWL SYC CCH AC.

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RESEARCH ARTICLE

National Trend and Characteristics of Acute Hepatitis C among HIV-Infected Individuals: A Matched Case-Control Study—Taiwan, 2001–2014

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Abstract

Background

Hepatitis C virus (HCV) infection has been increasingly recognized among HIV-infected men who have sex with men (MSM) worldwide. We investigated the trend of and factors associated with acute hepatitis C (AHC) among HIV-infected individuals in Taiwan.

Methods

The National Disease Surveillance System collects characteristics of AHC, HIV, syphilis, and gonorrhea cases through mandatory reports and patient interviews. Reported AHC patients in 2014 were interviewed additionally on sexual and parenteral exposures. Information on HCV genotypes were collected from the largest medical center serving HIV-infected Taiwanese. We defined an HIV/AHC case as a documented negative HCV antibody test result followed within 12 months by a positive test in a previously reported HIV-infected individual. Each case was matched to two HIV-infected, non-AHC controls for age, age of HIV diagnosis, sex, transmission route, HIV diagnosis date, and county/city. Conditional logistic regression was used to identify associated characteristics.

Results

During 2001–2014, 93 of 6,624 AHC reports were HIV/AHC cases; the annual case count increased from one in 2009 to 34 in 2014. All were males (81 [87%] MSM) aged 21–49 years with AHC diagnosed 2–5,923 days after HIV diagnoses. Sixty-eight (73%) lived in the Taipei metropolitan area. Detected HCV genotypes were 2a (n = 6), 1b (n = 5), 1b + 2a (n = 1) and 2b (n = 1). Among 28 HIV/AHC patients interviewed in 2014, 13 (46%) reported engaging in unprotected sex ≤3 months before AHC diagnosis. Seventy-nine HIV/AHC cases were matched to 158 controls. HIV/AHC was associated with recent syphilis

Competing Interests: The authors have declared that no competing interests exist.

(adjusted odds ratio [aOR], 10.9; 95% confidence interval [CI], 4.2–28.6) and last syphilis >6 months (aOR, 2.9; 95% CI, 1.2–6.9).

Conclusions

HIV/AHC cases continued to increase particularly among sexually active HIV-infected MSM with a syphilis diagnosis in northern Taiwan. We recommend surveillance of associated behavioral and virologic characteristics and HCV counseling and testing for HIV-infected men in Taiwan.

Introduction

Hepatitis C virus (HCV) infects an estimated 2.6%–3.1% of the world population and is a leading cause of liver disease [1].Without treatment, approximately 80% of patients with acute HCV infection progress to develop chronic infection and are at long-term risk for cirrhosis, hepatocellular carcinoma, and decompensated liver disease [2]. In HIV-infected individuals, HCV coinfection is common and the seroprevalence ranges from <5% in low-risk patients, 5–10% among MSM, to 50–90% among injection drug users (IDU) [3]. In HIV/HCV-coinfected individuals, HCV disease progresses more rapidly than HCV-monoinfected individuals and has become a leading cause of non-AIDS death [4–11].

Although HCV is primarily transmitted parenterally and sexual transmission of HCV in heterosexuals is considered rare [12], outbreaks of acute hepatitis C (AHC) through sexual transmission have been increasingly recognized among HIV-infected and HIV-uninfected men who have sex with men (MSM) in Europe, North America, and Australia [13–28]. Factors associated with sexually-transmitted AHC included unprotected receptive anal sex, use of sex toys, mucosally-administered recreational drug use (particularly methamphetamine use during sex), and concurrent or recent mucosally ulcerative sexually transmitted diseases (STDs) [29]. HIV-infected MSM carry approximately 4 times the risk of acute HCV acquisition than HIV-uninfected MSM, likely attributed to increased HCV susceptibility from HIV-induced compromise of humoral and cellular immunity [30–32].

In Asia, epidemiology of AHC among HIV-infected individuals has only been recently described in two hospital-based cohorts, one in Taiwan and the other in Japan [33–35]. Both studies demonstrated an increasing incidence of HCV seroconversion or AHC among HIV-infected MSM [33–34]. In the Taiwanese cohort, HCV seroconversion within 3 years was identified in 30 HIV-infected individuals (93.3% MSM) during 2001–2010 and was associated with recent syphilis acquisition [33]. In the Japanese cohort, AHC was diagnosed in 35 HIV-infected individuals during 2001–2012 who were predominantly MSM (96.6%) and antiretroviral therapy (ART)-experienced (90.6%) with well-suppressed plasma HIV RNA load (PVL) [35]. However, these studies are limited by the single-center design and the findings might not be generalized to HIV-infected individuals nationwide. We aimed to use the national surveillance data to investigate the trend of and factors associated with AHC among HIV-infected individuals in Taiwan.

Materials and Methods

Surveillance of AHC

Since 1999, the Communicable Disease Control Act in Taiwan has mandated healthcare providers to notify local public health departments of AHC cases within 7 days of diagnoses based on clinical assessment and commercially available HCV screening tests (generally enzyme

immunoassays [EIA]). Healthcare providers are required to report demographic, clinical, and laboratory characteristics of AHC cases through the web-based, Taiwan Centers for Disease Control (TCDC)-operated Notifiable Disease Surveillance Systems (NDSS).

As a guidance to healthcare providers, TCDC has offered clinical and laboratory criteria for AHC reporting. During 1999–2014, the clinical criteria included the following conditions in an individual with a positive HCV antibody test result: (1) clinical presentations consistent with acute hepatitis; (2) a serum alanine aminotransferase (ALT) level $\geq$100 IU/L (included since July 1, 2006); (3) exclusion of chronic HCV infection and non-HCV causes of acute hepatitis; and (4) jaundice (included during November 2010–March 2014). The laboratory criteria included the following conditions: (1) documented HCV seroconversion, defined as a documented negative HCV antibody test result followed within 12 months by a positive test; and (2) a positive nucleic acid test for HCV RNA with a negative HCV antibody (included after March 6, 2014).

TCDC recommends reporting of AHC if a case meets all of the clinical criteria or any of the laboratory criteria. However, in practice the NDSS has accepted all AHC reports from healthcare providers even if the criteria are not fully met. The NDSS has also accepted cases with a positive HCV antibody test result and ALT level $\geq$100 IU/L with or without documented HCV seroconversion from blood donation centers. Notably, because excessive reporting of HCV EIA-positive cases from blood donation centers in 2013 (341 cases in 2013 compared with 32–83 cases annually during 2009–2011) raised concerns about false-positive results which was considered more often in blood donors [36], TCDC has recommended blood donation centers conduct recombinant immunoblot assay (RIBA) in HCV EIA-positive cases before reporting since 2014.

The NDSS further classified reported cases based on the TCDC's AHC case definitions which had undergone 7 revisions during 1999–2014. Varied AHC case definitions have been used in published studies, such as HCV seroconversion $\leq$6 months or $\leq$12 months, or ALT level >100 IU/L followed by HCV seroconversion [33, 35, 37]. Because the NDSS readily collected data on HCV seroconversion within 12 months, for this study, we chose to ignore the NDSS case classification but follow the AHC case definitions established by the European AIDS Treatment Network [38]. Accordingly, we defined an HIV/AHC case as a documented negative HCV antibody test result followed within 12 months by a positive test (documented HCV seroconversion) in a previously reported HIV-infected individual. Because information on documented HCV seroconversion had not been collected until June 2001, only AHC cases in the NDSS reported during June 2001–December 2014 were analyzed to identify HIV/AHC cases.

The NDSS started to collect data on qualitative HCV RNA test results (positive, negative, or not conducted) among AHC cases from reporting healthcare facilities after March 6, 2014. Because the NDSS did not require physicians to report HCV viral load and genotypes among AHC cases, we collaborated with the National Taiwan University Hospital (NTUH), the largest medical center serving HIV-infected patients in the Taipei metropolitan area, to retrospectively collect data on HCV viral load and genotypes among HIV/AHC patients who received medical care at NTUH.

Survey of sexual and parenteral exposures before AHC diagnosis

During March 7–December 31, 2014, a standardized questionnaire survey was administered by local health department staff through telephone interviews in all AHC cases with documented HCV seroconversion. Patients were asked about engagement in unprotected heterosexual or same-sex sex and the number of sex partners $\leq$3 months before AHC diagnosis, and parenteral exposures $\leq$6 months before AHC diagnosis.

Surveillance of HIV infection, syphilis, and gonorrhea

In Taiwan, TCDC has mandated medical professionals to notify local public health departments of cases of HIV infection within 24 hours of diagnoses since 1985, and of syphilis and gonorrhea within 7 days of diagnoses since 1999. Surveillance of these diseases through the NDSS has been described in details [39]. In all reported HIV cases, self-reported information on sexual behaviors, injection drug use, and other risk factors is collected through face-to-face public health interviews. The NDSS also collected the patients' CD4 count, PVL, and ART use every 3–6 months.

Database linkage

To identify HIV/AHC cases and reports of syphilis and gonorrhea before AHC diagnoses, the NDSS databases of AHC (June 2001–2014), HIV infection (1985–2014), syphilis (1999–2014), and gonorrhea (1999–2014) were linked using a government-issued, non-duplicated national identification number that is unique and compulsory for each Taiwanese national.

We defined the Taipei metropolitan area as the jurisdiction of Taipei City and New Taipei City, and defined other metropolitan areas as the jurisdiction of cities of Keelung, Taoyuan, Hsinchu, Taichung, Chiayi, Tainan, and Kaohsiung. The other jurisdictions were defined as non-metropolitan areas.

1:2 Matched case-control study

We conducted a 1:2 matched case-control study to identify risk factors for AHC among HIV-infected individuals. Each HIV/AHC case was matched to two HIV-infected, non-AHC controls in the NDSS database on age (+/-5 years), age at HIV diagnosis (+/-5 years), sex, mode of transmission (MSM, heterosexual contact, and IDU), date of HIV diagnosis (+/-30 days), and county/city of residence at HIV diagnosis. If a case-patient could not be matched for two controls, the case-patient is excluded from the analysis. If more than two subjects could be identified as controls for a case-patient, we selected the subjects whose age at HIV diagnosis best matched the case-patient. Because IDUs had a high prevalence of chronic HCV infection but the NDSS did not collect chronic HCV data, we excluded IDUs from the case-control study to reduce the possibility of matching HCV-infected controls.

We defined the observation period for a case-patient and controls in each pair as the interval between HIV diagnosis and AHC diagnosis of the case-patient. The end of observation was defined as the case-patient's date of AHC diagnosis. To ensure a similar observation period in each pair, subjects were not eligible as controls if they died before the date of the corresponding case-patients' AHC diagnosis. Information on previous reporting of syphilis and gonorrhea, and the last CD4 count, PVL, and ART use by the end of the observation was collected from NDSS. Recent syphilis and recent gonorrhea were defined as any report of syphilis and gonorrhea $\leq$6 months before the end of observation, respectively.

Statistical analysis

The trend in proportions was evaluated with the chi-square test for trend. Cases were mapped by using the software Quantum GIS version 1.7.4. We conducted bivariate analyses to compare characteristics of case-patients and controls using the chi-square test or Fisher's exact test for categorical variables and the Wilcoxon rank sum test for noncategorical variables. All comparisons were two-tailed and a p-value <0.05 was considered significant. Variables that were associated with AHC ($p<0.2$) in bivariate analyses were considered candidates in a conditional logistic regression model. Adjusted odds ratio (aOR) and 95% confidence intervals were

calculated. The analyses were conducted with the statistical software package SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina).

Ethics statement

Data obtained for the NDSS was for public health surveillance purposes. This study was approved by the Institutional Review Board of TCDC. Informed consent was not obtained because the data used in this study was analyzed anonymously.

Results

Of 6,624 reported AHC cases to TCDC through the NDSS during June 2001–December 2014, 2,395 (36.2%) had documented HCV seroconversion, of which 93 (3.9%) met the case definitions of HIV/AHC.

Trend of HIV/AHC

The first HIV/AHC case was reported in April 2005, and 5 HIV/AHC cases (three IDUs, one heterosexual, and one MSM) were reported during 2006–2007. The annual number of HIV/AHC cases consistently increased from one case in 2009 to 34 cases in 2014 (Fig 1). Of

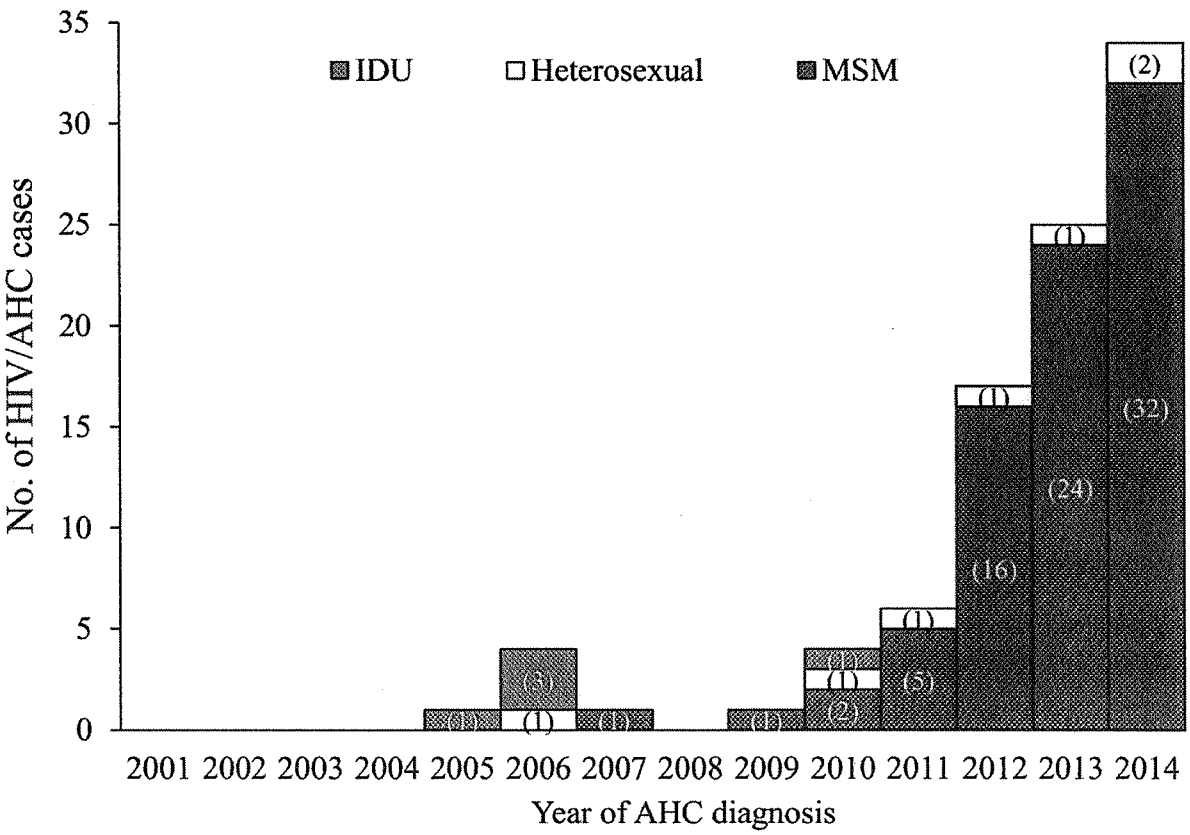


Fig 1. Trend of HIV/AHC cases by population at risk, Taiwan, 2001–2014. [a]Numbers in parentheses indicate numbers of HIV/AHC cases in each risk category.

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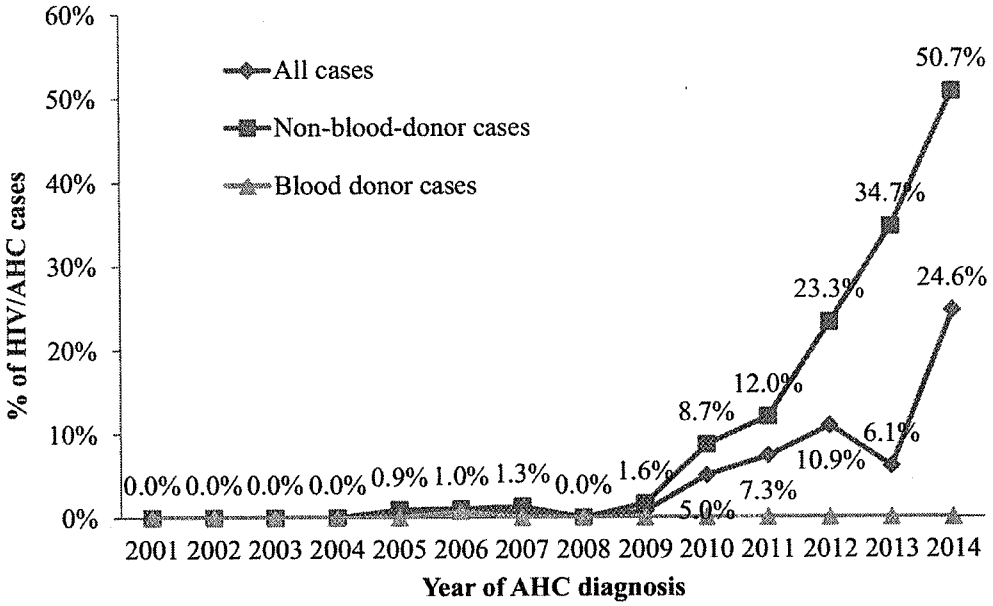


Fig 2. Proportion of HIV/AHC cases among AHC cases with documented HCV seroconversion, Taiwan, 2001–2014.

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87 HIV/AHC cases reported during 2009–2014, MSM, heterosexuals, and IDU accounted for 80 (92%), 6 (6.9%), and one (1.1%) cases, respectively.

The proportion of HIV/AHC cases among AHC cases with documented HCV seroconversion reported annually was 0.6–0.8% during 2005–2007 and increased from 0.8% in 2009 to 24.6% in 2014 but was inconsistently lower (6.1%) in 2013 (Fig 2). To remove confounding of excessive reporting from blood donation centers in 2013, we separately analyzed blood-donor cases and non-blood-donor cases. Of 1,445 blood-donor cases with documented HCV seroconversion, three (0.2%) were HIV/AHC cases (two IDUs and one heterosexual), all reported in 2006. Of 950 non-blood-donor cases with documented HCV seroconversion, the proportion of HIV/AHC cases reported annually was overall 0.9%–1.3% during 2005–2007 and consistently increased from 1.6% in 2009 to 50.7% in 2014 ($p<0.001$) (Fig 2).

Characteristics of HIV/AHC cases

Of 93 HIV/AHC cases reported during June 2001–December 2014, all were males with a median age of 34 years (range, 21–49) at the time of AHC diagnosis. HIV diagnosis was made within a median of 1,467 days (range, 2–5,923) before AHC diagnosis. Fig 3 showed geographic distribution of 93 HIV/AHC cases. The majority of HIV/AHC patients were MSM (87%), residents in the Taipei metropolitan areas (73%), and not hospitalized (90%) at the time of AHC diagnosis (Table 1). Approximately half (49%) had symptoms compatible with acute hepatitis, and 82% had an elevated ALT level ≥100 IU/L (median, 206; range, 100–1,808). Syphilis and gonorrhea had been reported in 77% and 20% of the HIV/AHC cases, respectively. Of the HIV/AHC cases with a previous syphilis or gonorrhea report, the median interval from the last reports of syphilis and gonorrhea to AHC diagnosis was 431(range, 0–3,005) and 623 days (range, 104–4,958), respectively.

Of 93 HIV/AHC cases, the median CD4 count was 519 cells/mm^3 (range, 27–1208; 20 [22%] with CD4 count <350 cells/mm^3) and the median PVL was undetectable (range, <20 to

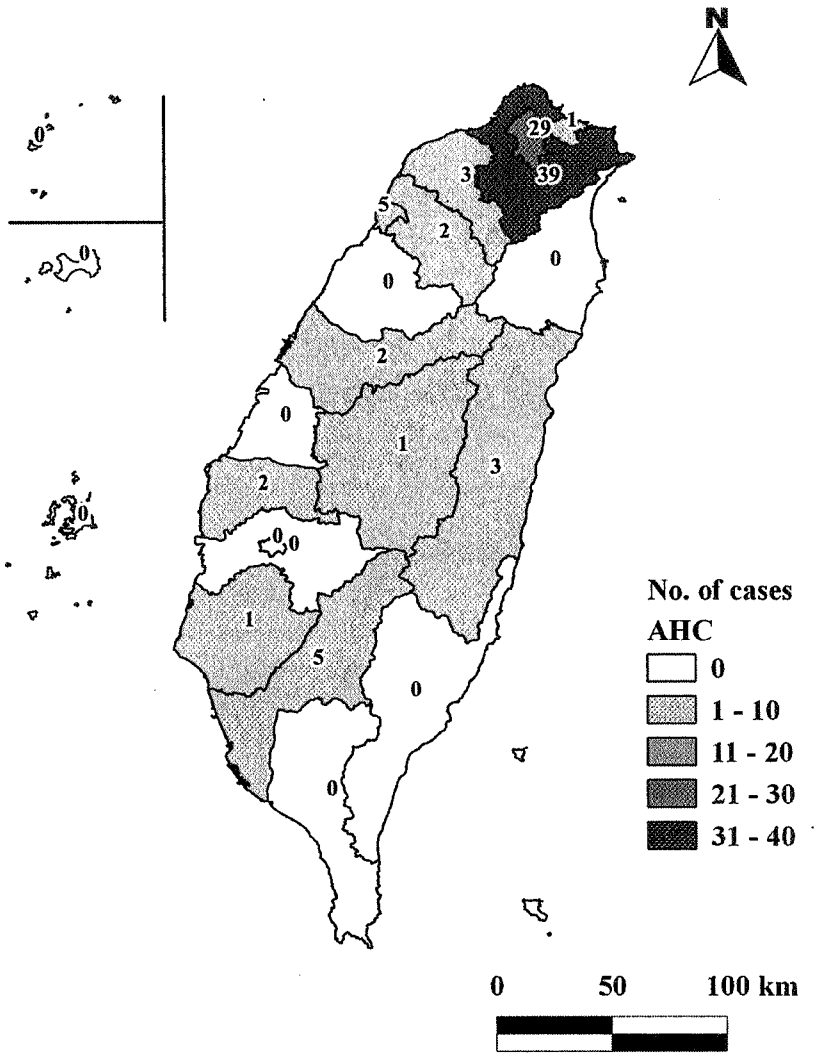


Fig 3. Number of HIV/AHC cases in each county and city, Taiwan, 2001–2014 [a]Numbers in the map indicate numbers of HIV/AHC cases in each city or county. This map is made by using the basemap provided by Taiwan Ministry of Interior under the Open Government Data License.

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1.2 x 10^6 copies/ml; 63 [68%] with a PVL <400 copies/ml) at the time of AHC diagnosis, and 70 (75%) had received ART before AHC diagnosis.

Of 16 HIV/AHC cases with available HCV virologic data, all were MSM with AHC diagnosed during 2012–2014 and HCV viral load were detectable in 15 (94%) with the median HCV viral load of 5.7 x 10^6 copies/mL (range, 2.8 x 10^3–1.2 x 10^8). Two patients were not tested for HCV genotypes because they lost to follow up. HCV genotype data were available in 13 patients. Detected genotypes were 2a (n = 6), 1b (n = 5), 1b + 2a (n = 1) and 2b (n = 1) (Table 2).

Of 31 HIV/AHC cases reported between March 6 and December 31, 2014, HCV RNA testing results were positive in 14 cases, not conducted in 16 cases, and missing in one case. No HIV/AHC case was reported as HCV RNA negative.

Sexual and parenteral exposures before AHC diagnosis

Of 31 HIV/AHC patients reported during March 7–December 31, 2014, 28 (90%) completed telephone interviews, two declined, and one was not contactable. Of 28 HIV/AHC patients successfully interviewed, 26 (93%) were self-reported MSM and two were heterosexuals. Of 26 MSM with HIV/AHC, 12 (46%) reported engagement in unprotected sex with a median of 1.5 male partners (range, 1–10) and one reported unprotected sex with a female partner ≤3 months before AHC diagnosis. The two heterosexuals with HIV/AHC denied recent unprotected sex. Nine (32%) of 28 HIV/AHC patients reported parenteral exposures ≤6 months before AHC diagnosis reported in, including intravenous or intramuscular injection for health reasons (n = 4), dental procedures (n = 3), surgery (n = 2), and sharing shavers or toothbrushes (n = 1) (Table 3).

1:2 Matched case-control study

Of 88 HIV/AHC, non-IDU cases reported during June 2001–December 2014, 79 cases (all MSM) were successfully matched to 158 HIV/non-AHC controls, all of which were reported during 2007–2014. Of 9 unsuccessfully matched cases, we identified only one matched control for 7 cases (5 heterosexuals and 2 MSM) individually and no matched controls for the other 2 heterosexual cases. Table 4 summarized comparisons of characteristics between HIV/AHC cases and matched HIV/non-AHC controls. In bivariate analysis, HIV/AHC cases were significantly associated with a higher last CD4 count and a syphilis report during the observation period (Table 4). In the conditional logistic regression model, factors independently associated with HIV/AHC were having the last syphilis report ≤6 months (recent syphilis) (aOR, 9.9; 95% CI, 3.9–25.2) and having the last syphilis report >6 months (aOR, 2.8; 95% CI, 1.2–6.5) during the observation period (Table 5).

Table 1. Characteristics of HIV-infected individuals with acute hepatitis C—Taiwan, 2001–2014

Characteristics at AHC diagnosis	HIV/AHC (N = 93) No. (%)
Age group, years	
21–30	36 (39)
31–40	39 (42)
41–50	18 (19)
Male sex	93 (100)
Mode of HIV transmission	
Male–male sex	81 (87)
Heterosexual	7 (8)
Injection drug use	5 (5)
Area of residence	
Taipei metropolitan area	68 (73)
Other metropolitan areas	17 (18)
Non-metropolitan areas	8 (9)
Symptoms compatible with acute hepatitis	46 (49)
Serum aminotransferase level ≥100 IU/L	76 (82)
Hospitalized	9 (10)
Previous syphilis report	72 (77)
Previous gonorrhea report	19 (20)

Abbreviations: AHC, acute hepatitis C; HIV, human immunodeficiency virus

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Table 2. HCV virologic characteristics of 16 HIV-infected individuals with acute hepatitis C at the National Taiwan University Hospital—Taiwan, 2009–2014.

Patient No.	AHC Diagnosis Year	HCV viral load (copies/mL)	HCV genotype
1	2012	3.8×10^8	1b
2	2013	5.7×10^6	Not tested (loss to follow up)
3	2013	6.6×10^6	2b
4	2013	2.8×10^3	2a
5	2013	1.7×10^7	1b
6	2013	1.7×10^5	1b + 2a
7	2013	3.5×10^4	1b
8	2013	1.2×10^8	2a
9	2013	2.7×10^4	2a
10	2014	1.6×10^7	1b
11	2014	7.2×10^5	2a
12	2014	1.5×10^5	1b
13	2014	3.3×10^5	2a
14	2014	1.8×10^7	Not tested (loss to follow up)
15	2014	3.7×10^7	2a
16	2014	Undetectable	NA

Abbreviations: AHC, acute hepatitis C; HIV, human immunodeficiency virus; HCV, hepatitis C virus; NA, not available.

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To examine if the 9 unsuccessfully matched cases could make a difference to the results, we included all 88 HIV/AHC, non-IDU cases and 165 matched controls in an unconditional logistic regression model. The results still showed that factors independently associated with HIV/AHC were recent syphilis (aOR, 8.9; 95% CI, 3.9–20.3) and having the last syphilis report >6 months (aOR, 2.2; 95% CI, 1.1–4.5) during the observation period.

Table 3. Sexual and parenteral exposures before diagnosis of acute hepatitis C among HIV-infected individuals—Taiwan, March 7–December 31, 2014.

Exposures associated with HCV acquisition before AHC diagnosis	HIV/AHC (N = 28)
	No. (%)
Unprotected sex ≤3 months	13 (46)
Sex partner of same-sex	12 (42)
No. of sex partners ≥2	6 (21)
Parenteral exposures ≤6 months	9 (32)
Injection for health reason	4 (14)
Dental procedures	3 (11)
Surgery	2 (7)
Shaver/toothbrush sharing	1 (4)
Needle-sharing	0 (0)
Blood component therapy	0 (0)
Hemodialysis	0 (0)
Acupuncture/blood-letting	0 (0)
Occupational needlestick injury	0 (0)
Tattoo/body-piercing	0 (0)

Abbreviations: AHC, acute hepatitis C; HIV, human immunodeficiency virus

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Table 4. Characteristics of HIV/AHC cases and matched HIV/non-AHC controls.

Characteristics during observation period	Cases (N = 79)	Controls (N = 158)	*P* Value
Age (years) [median (IQR)]	32 (28–38)	32 (28–38)	0.81
Age at HIV diagnosis (years) [median (IQR)]	28 (24–32)	28 (24–32)	0.78
Year of HIV diagnosis [no. (%)]			
1996–2002	10 (13)	20 (13)	1.00
2003–2008	29 (37)	58 (37)	
2009–2014	40 (51)	80 (51)	
Observation period (months) [median (IQR)]	52 (23–87)	52 (23–88)	1.00
Last CD4 count (cells/mm^3) [median (IQR)]	525 (209–707)	466 (346–592)	0.02
Last CD4 count (cells/mm^3) [no. (%)]			
<200	0 (0)	6 (4)	0.15
200–349	16 (20)	37 (23)	
350–499	20 (25)	48 (30)	
≥500	43 (54)	67 (42)	
Last PVL (log_{10} copies/ml) [median (IQR)]	1.3 (1.3–3.5)	1.3 (1.3–3.8)	0.68
Last PVL (log_{10} copies/ml) [no. (%)]			
<400	56 (71)	107 (68)	0.62
≥400	23 (29)	51 (32)	
Antiretroviral-experienced [no. (%)]	63 (80)	115 (73)	0.24
Previous syphilis report [no. (%)]			
Last report ≤6 months (recent syphilis)	31 (39)	16 (10)	<0.001
Last report >6 months	32 (41)	64 (41)	
None	16 (20)	78 (49)	
Previous gonorrhea report [no. (%)]			
Last report ≤6 months	2 (3)	1 (1)	0.09
Last report >6 months	12 (15)	12 (8)	
None	65 (82)	145 (92)	

Abbreviations: AHC, acute hepatitis C; HIV, human immunodeficiency virus; IQR, interquartile range; PVL, plasma viral load

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Discussion

To our knowledge, this is the first study that describes HIV/AHC epidemiology based on national surveillance data. The findings demonstrate a rapidly increasing trend of HIV/AHC

Table 5. Characteristics associated with HIV/AHC in conditional logistic regression.

Characteristics during observation period	Adjusted OR (95% CI)	*P* Value
Last CD4 count (per 100 cells/mm^3 increase)	1.1 (0.98–1.3)	0.08
Previous syphilis report		
Last report ≤6 months (recent syphilis)	9.9 (3.9–25.2)	<0.001
Last report >6 months	2.8 (1.2–6.5)	0.01
None	Reference	Reference
Previous gonorrhea report [no. (%)]		
Last report ≤6 months	1.9 (0.7–5.0)	0.19
Last report >6 months	5.2 (0.4–60.0)	0.19
None	Reference	Reference

Abbreviations: AHC, acute hepatitis C; HIV, human immunodeficiency virus; OR, odds ratio; CI, confidence intervals

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in Taiwan since 2009, characterized by HIV-infected MSM as the major affected population. HIV/AHC was predominantly identified in MSM aged 21–40 years who resided in the Taipei metropolitan area. Risk factors include recent unprotected sex and multiple sex partners, and a past history of syphilis, particularly recent syphilis, is associated with AHC among HIV-infected individuals. These results are consistent with the results of a prior study that first reported increased incidence of recent HCV infection during 2006–2010 in a MSM-predominant HIV cohort in the Taipei metropolitan area [33]. In that study, HCV acquisition within 3 years before enrollment was also associated with recent syphilis (≤6 months) but behavioral risk factors were not explored. Our study applies the European AIDS Treatment Network AHC case definitions, HCV seroconversion within 12 months [38], and the findings confirm that AHC continues as an emerging STD among HIV-infected MSM nationwide and is associated with behavior factors indicating increased sexual risks.

We also demonstrate MSM has predominated over IDU as the major population at risk for HIV/AHC in Taiwan. The findings coincide with a changing epidemiology of HIV infection domestically. In the early 2000s, HCV seroprevalence in Taiwan was 41% for IDUs, 5–9% for female prostitutes, and 4% for male prostitutes [40]. During 2004–2008, a large HIV epidemic was identified among IDUs, characterized by extremely high HCV seroprevalence (>96%) and parenteral exposures including sharing needles, syringes, diluent, and drug paraphernalia [41–44]. Prompt implementation of harm reduction programs that provided IDUs with free clean syringes and methadone led to rapid decline of new HIV cases after 2006 [45]. MSM have become the most preferentially affected population for HIV infection since 2008 [46]. Domestic studies showed HCV seroprevalence (5.5%) and incidence among HIV-infected MSM increased during 2006–2010, whereas HCV seroprevalence remained low (1.1%) among HIV-negative MSM [30, 47]. The alarming upsurge in AHC among HIV-infected MSM suggest that sexual transmission, particularly male–male sex, has recently replaced injection drug use as the major route of HCV transmission among HIV-infected individuals in Taiwan. Recently a 2.2-foldincrease of HCV incidence from 2006–2013 was reported among voluntary counseling and testing clients in Taiwan [48]. Whether HCV infection has increased among sexually active HIV-negative populations warrants further investigation.

The predominant HCV genotypes (2a and 1b) during 2012–2014 identified in the limited sample of this study were identical with the HCV genotypes reported earlier among HIV-infected MSM from the same medical center but different from the predominant HCV genotypes (1a, 6a, and 3a) among IDUs in Taiwan, indicating independent HCV transmission networks in these two populations [30, 41, 48]. Phylogenetic analysis would help determine whether continued or clustered HCV transmission among HIV-infected MSM has occurred across the two study periods. Whereas HCV genotypes 3a and 6a were identified in the previous study, we identified a case infected with HCV genotype 2b, a genotype that was not previously reported among MSM with HIV/AHC in Taiwan but recently reported in Japan associated with non-spontaneous HCV clearance [35]. Surveillance efforts are needed to monitor circulation of HCV genotype 2b and the impact on spontaneous HCV clearance rate among HIV-infected MSM.

The association of AHC with syphilis in our study supports the contributing role of ulcerative STDs to HCV acquisition through impairing mucosal integrity [14, 18, 19, 28, 29, 33, 49]. The increasing HIV/AHC cases and incidence might have been driven by the recently demonstrated rising incidence of syphilis among HIV-infected young Taiwanese men (aged 15–34 years) [50]. A recent study on HIV-infected MSM in Taipei metropolitan areas also concluded association of syphilis with HIV serosorting and recreational drug use, suggesting the facilitating roles of these behavioral factors for STD acquisition [51]. On the other hand, the disproportionate geographic predilection for HIV/AHC in Taipei metropolitan area can be only

partially explained by the estimates that 37% of HIV-infected Taiwanese resided in Taipei City and New Taipei City [52]. Regional difference in risky behaviors might play a more important role, as exemplified by an internet-based survey in 2012 that showed MSM in northern Taiwan used recreational drugs and engaged in drug-fueled unprotected anal sex more commonly than MSM in central or southern Taiwan [53]. Notably, although studies in the Western countries have associated HIV/AHC with methamphetamine use among MSM, the 2012 survey indicated that MSM in southern Taiwan used amphetamine more commonly than MSM in northern or central Taiwan [53]. The reasons for such divergence remained unstudied; our anecdotal observation suggested that cheaper cost of domestically produced methamphetamine in southern Taiwan and relative preference of nontraditional imported recreational drugs in northern Taiwan might be contributing factors. Comprehensive investigations of sexual behaviors and recreational drug use among HIV/AHC patients, taking regional differences into account, are recommended to provide guidance for risk assessment and public health interventions against HCV transmission among HIV-infected MSM.

Although the national HIV treatment guidelines have recommended testing of liver function every 6 months and HCV antibody every 12 months for HIV-infected individuals since 2010, adherence to guidelines was inadequate and HCV testing among HIV-infected individuals has been generally triggered by elevated ALT levels [54]. However, ALT-triggered HCV testing strategy might lead to missing 21% of HIV/AHC cases [55]. A recent study using a Monte Carlo computer simulation model demonstrated 6-month ALT and 12-month HCV antibody testing a cost-effective screening strategy for AHC among HIV-infected MSM when the HIV incidence was $\leq$1.25 cases/100 person-years [56]. Because the NDSS did not collect data on chronic HCV infection, we were not able to have a correct denominator for estimating incidence of AHC among HIV-infected individuals. However, a recent single-center study with service populations in the Taipei metropolitan area estimated that the incidence of recent hepatitis C among HIV-infected MSM was 1.23 per 100 person-years, an incidence level in support of the currently endorsed screening strategy (6-month ALT and 12-month HCV) [30, 56]. Given the regional variation in the number of AHC cases, prospective or retrospective cohort studies that collect baseline and follow-up data on HCV infection to determine the incidence of HIV/AHC nationwide would provide evidence to inform the appropriate screening strategy for AHC among HIV-infected populations at the national and regional levels.

This study is subject to at least the following limitations. First, because our case definitions require documented HCV seroconversion within 12 months, the surveillance data is expected to underestimate AHC disease burden and overrepresent HIV-infected individuals because of enhanced access and frequency of HCV screening among this population. Furthermore, decreasing AHC case detection over the study period is expected because ALT levels and jaundice were added into the clinical criteria of reporting after 2006 and 2010, respectively. Given the limitations, the fact that we still demonstrate an increasing trend of HIV/AHC suggests there is true upsurge of case numbers and the detected cases are only the tip of the iceberg. Second, MSM status and behavioral data were self-reported, collected through public health interviews, and thus subject to socially-desirable biases that could lead to underreported male–male and unprotected sex. Third, our surveillance system depends exclusively on HCV antibody tests before March 6, 2014, not supplemented by RIBA or RNA results in positive AHC cases other than blood donors. Such screening strategy has been reported to have <5%–60% false-positive rates among varied populations [36]. However, the zero HCV RNA negative result among HIV/AHC cases after the NDSS started to collect HCV RNA data in March, 2014 suggested that false-positive results might be rare in our sample. Finally, misclassification might have occurred because we did not have data on chronic HCV infection among the control

subjects, leading to underestimation of the crude and adjusted odds ratios for the associated factors.

We recommend health authorities in Taiwan continue surveillance of the trend of HIV/AHC and work with public and private sectors to promote awareness of AHC among HIV-infected men. Healthcare providers should maintain a high index of suspicion of AHC and provide HCV counseling and testing for HIV-infected men with a past history of syphilis, particularly those with recent syphilis.

Author Contributions

Conceived and designed the experiments: YCL HYS CCH. Performed the experiments: YCL JHC. Analyzed the data: YCL. Contributed reagents/materials/analysis tools: YCL MST JHC. Wrote the paper: YCL MST HYS CCH JHC.

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Increasing incidence of recent hepatitis C virus infection among persons seeking voluntary counselling and testing for HIV and sexually transmitted infections in Taiwan

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ABSTRACT

Objectives: The incidence of hepatitis C virus (HCV) infection among HIV-negative men who have sex with men (MSM) is rarely investigated in the Asia-Pacific region. We aimed to estimate the incidence rate of and factors associated with recent HCV infection among the clients seeking voluntary counselling and testing (VCT) services for HIV in Taiwan.

Methods: During 2006–2013, 12 143 clients sought VCT services for HIV. Clients with subsequent follow-up tests at an interval of 6 months or longer were included to estimate the incidence rate of HCV seroconversion. Phylogenetic analysis of HCV sequences from VCT clients and HIV-positive patients was performed.

Results: The overall HCV seroprevalence at baseline was 0.3%. Of 2150 clients testing negative for anti-HCV antibody at baseline with a total of 5074.99 person-years of follow-up (PYFU), 17 (0.8%) developed HCV seroconversion, leading to an overall incidence rate of 3.35 per 1000 PYFU (95% CI 1.76 to 4.94), which increased from 2.28 (95% CI 0.05 to 4.51) in 2006–2009, to 3.33 (95% CI 0.86 to 5.80) in 2010 to 2011 and 4.94 per 1000 PYFU (95% CI 0.99 to 8.99) in 2012–2013; the incidence of early syphilis increased from 11.91 to 13.28 and 31.78 per 1000 PYFU in the three corresponding periods. In multivariate analysis, having HIV-positive partners (adjusted HR (AHR) =3.756; 95%CI 1.180 to 11.955) and developing a rapid plasma reagin titre of 4 or greater (AHR=9.978; 95% CI 1.550 to 64.233) were significantly associated with HCV seroconversion.

Conclusions: An increasing trend of recent HCV infection occurs among individuals seeking VCT services in Taiwan. Having HIV-positive partners and having syphilis are independently associated with recent HCV seroconversion.

Strengths and limitations of this study

- This is the first study in the Asia-Pacific region to estimate hepatitis C virus (HCV) incidence among individuals seeking voluntary counselling and testing services, particularly HIV-negative men who have sex with men (MSM).
- This study demonstrates the increasing incidence of HCV infection in high-risk HIV-negative MSM and provides useful information to clinical physicians.
- Owing to the small number of cases and single hospital study, more surveillance studies are warranted.

INTRODUCTION

After the introduction of combination antiretroviral therapy, AIDS-related morbidity and mortality have decreased significantly, and end-stage liver disease has become the leading cause of non-AIDS-related deaths in the HIV-positive population, especially among those co-infected with hepatitis C virus (HCV).[1] While parenteral transmission remains the most efficient route of HCV infection,[2] sexual contact has not been considered as an important route for HCV transmission.[3] In the USA and Western Europe, HCV seroprevalence is 72–95% among HIV-positive persons with a history of injection drug use, 1–12% in HIV-positive men who have sex with men (MSM) and 9–27% in HIV-positive heterosexuals.[4] In Taiwan, HCV seroprevalence is 96.8% among HIV-positive persons with a history of injection drug use, 5.5% in HIV-positive MSM and 10.9% in HIV-positive heterosexuals.[5–8]

Since 2000, outbreaks of acute HCV infection among HIV-positive MSM without a history of injection drug use have been increasingly reported,[9–12] with incidence rates ranging from 1–3 per 1000 person-years

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(PY) to 10 or more per 1000 PY.[13–18] Several factors have been identified to be associated with acquisition of HCV infection via sexual contacts among HIV-positive MSM, such as unprotected receptive anal intercourse with multiple partners and syphilis, rectal trauma with bleeding, fisting and use of non-parenteral recreational drugs.[19–22]

However, there are limited data about the incidence of HCV infection among HIV-negative MSM, especially in the Asia-Pacific region. In the UK, unselected screening of HCV among MSM over a 6-month period when they attended sexual health clinics showed similar HCV seroprevalence between the general UK population (0.72%) and MSM with HIV-negative or unknown HIV status (0.61%).[23] However, in a study by Richardson *et al*,[15] the incidence of HCV infection in HIV-negative MSM increased from 0 per 1000 PY in 2000–2003 to 5.8 per 1000 PY in 2006.

Our previous study in Taiwan has shown an increasing trend of recent HCV infection among HIV-positive MSM between 2000 and 2010, which was associated with syphilis.[18] In this study, we aimed to estimate the incidence rate of recent HCV infection among individuals who were not injecting drug users (IDUs), and who sought voluntary counselling and testing (VCT) services for HIV and sexually transmitted infections (STIs) at a university hospital in Taiwan. Preliminary results of this study were presented in the 2014 HIV Drug Therapy Glasgow Congress.[24]

METHODS

Study setting

VCT services for HIV and syphilis were offered free of charge in Taiwan, with grant support from Taiwan Centers for Disease Control.[25] Every client seeking VCT services would have a unique code for identification of test results, and complete an anonymous, self-administered questionnaire to obtain information on the demographics, sexual practices, risk behaviours for HIV infection, prior or current STIs, number of sexual partners, HIV serostatus of sexual partners, condom use and use of injection drugs or non-injecting recreational drugs. After completion of integrated pretesting and post-testing counselling, a blood sample was obtained for testing for HIV, syphilis and viral hepatitis.[25]

In this study, all clients who sought VCT services between May 2006 and December 2013 were included, and clients using the same code who sought VCT services more than two times with an interval of 6 months or longer were eligible for inclusion to estimate the incidence rate of HCV seroconversion. IDUs and clients who sought VCT services only once or with repeat tests performed at an interval of less than 6 months were excluded. The participants gave written informed consent by using the unique code.

Laboratory investigations

Antibodies to HCV were determined with a fourth-generation enzyme immunoassay (GB NANBASE C-96, V4.0). The HCV NS5B gene sequences, amplified from VCT clients with positive HCV antibody and patients co-infected with HIV and HCV in our cohort, between 2006 and 2013, were used in phylogenetic analysis. A 336-bp fragment covering partial HCV NS5B (nucleotides (nt) 8294–8629 relative to HCV reference strain H77) was amplified by PCR as previously described.[18] The final PCR products were sequenced. Phylogenetic analysis was performed to determine the HCV genotypes by the use of NS5B sequences amplified from HCV-positive patients in our cohort.[18 26] The study and reference sequences were aligned using the Clustal W program with minor manual adjustment. The tree was constructed by the neighbour-joining method based on the Kimura two-parameter distance matrix listed in MEGA software (V.3.0).[27]

Nucleotide sequence accession numbers

The GenBank accession numbers of the obtained partial NS5B HCV sequences used in the phylogenetic analysis were JQ060117–121, JQ060123–124, JQ060126, JQ060131–133, HM031199, HM031203–204, HM031211, DQ179117, DQ663604, DQ663608, DQ666268 and EU255966. The GenBank accession numbers for the four seroconversion sequences were 1847251, 1847258, and 1847261-62.

Definitions

Recent HCV seroconversion was defined as the first positive HCV antibody that was detected within 1 year after the previous negative HCV antibody. The date of seroconversion was arbitrarily assigned as the midpoint between the date of the last negative HCV antibody and that of the first positive HCV antibody.[18] Recent syphilis was defined as having an rapid plasma reagin (RPR) titre with a fourfold increase or new RPR seroreactivity within 6 months of HCV seroconversion or within 6 months of the last anti-HCV test.[18]

Statistical analyses

All statistical analyses were performed by SPSS software, V.17 (SPSS Inc, Chicago, Illinois, USA). Categorical variables were compared using χ^2 or Fisher's exact test whereas non-categorical variables were compared using Student's t test or Mann-Whitney U test. All tests were two-tailed and a p value <0.05 was considered significant. Cox proportional hazards model was used to identify factors associated with HCV seroconversion. All included clients were followed from the date of first VCT visit during the study period to the date of the last VCT visit or HCV conversion. To assess the trend of HCV infection, the follow-up duration was further categorised into three time periods, 2006–2009, 2010–2011 and 2012–2013. The incidence rate of HCV seroconversion in each study period was calculated as number of episodes of HCV seroconversion per 1000 PY of follow-up (PYFU). Poisson regression was used for comparisons of incidence rates in three time periods (2006–2009, 2010–2011 and

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2012–2013). A nested case–control study was also performed to compare the clinical characteristics of HCV seroconverters with those of HCV non-seroconverters. Every HCV seroconverter was matched with four non-seroconverters for age, gender, year of first VCT visit and duration of follow-up during the study period.

RESULTS

Between May 2006 and December 2013, 12 143 individuals sought VCT services and underwent a total of 18 246 tests, and 9986 clients were excluded in the further analysis because of being IDUs or having only one test during the period. The remaining 2157 clients contributed a total of 8260 tests with an interval of 6 months or longer between two tests.

At baseline, HCV seropositivity was found in seven clients (0.3%). Of the 2150 clients with negative HCV antibody at baseline who contributed a total of 5074.99 PYFU, 17 (0.8%) had HCV seroconverted during the follow-up. This led to an overall incidence rate of 3.35 per 1000 PYFU (95% CI 1.76 to 4.94). The clinical characteristics of the 17 HCV seroconverters and 2133 clients who remained seronegative for HCV are shown in table 1. Thirteen of the 17 (76.5%) HCV seroconverters were MSM and 4 (23.5%) were heterosexuals.

During the three study periods, the incidence rate of HCV seroconversion increased from 2.28 per 1000 PYFU (95% CI 0.05 to 4.51) in 2006–2009 and 3.33 per 1000 PYFU (95% CI 0.86 to 5.80) in 2010–2011, to 4.94 per 1000 PYFU (95% CI 0.99 to 8.99) in 2012–2013 (figure 1). The incidence rate of syphilis also increased from 11.91 per 1000 PYFU in 2006–2009 to 31.78 per 1000 PYFU in 2012–2013 (figure 1); the rates were significantly higher in HCV seroconverters than non-seroconverters during the three corresponding study periods.

In multivariate analysis, HCV seroconverters were more likely to have HIV-positive partners (adjusted HR (AHR) 3.756; 95% CI 1.180 to 11.955) and a RPR titre of four or greater (AHR 9.978; 95% CI 1.550 to 64.233) than HCV non-seroconverters (table 2). In the nested case–control study, the proportion of clients having HIV-positive sexual partners remained higher in the HCV seroconverters than in the non-seroconverters (23.5% vs 3.1%, p=0.016), so did the proportion of those who had recent syphilis (11.8% vs 0%, p=0.04) and an RPR titre of four or greater (11.8% vs 0%, p=0.04). During follow-up, HCV seroconverters tended to be more likely to become HIV positive than non-seroconverters (17.7% vs 3.1%, p=0.059) (see online supplementary table S1). In multivariate analysis, having HIV-positive sexual partners remained independently associated with HCV seroconversion with an adjusted OR of 6.931 (95% CI 1.064 to 45.163) (see online supplementary table S2).

Among the 17 HCV seroconverters, four clients had HCV viraemia detected by the PCR assay. One client was infected with HCV genotype 2a, and three with genotype 1b. Phylogenetic analysis was performed to determine their relationship with prevalent HCV sequences of HIV-positive MSM (n=11) and IDUs (n=9). The sequence of HCV isolates from HCV seroconverts among the VCT clients appeared to cluster with those from HIV-positive MSM, rather than those of IDUs (figure 2).

DISCUSSION

In this study, we demonstrate an increasing trend of HCV seroconversion among VCT clients who were not

Table 1 Clinical characteristics of HCV seroconverters and non-seroconverters

Characteristics	Seroconverters N=17 (%)	Non-seroconverters N=2133 (%)	p Value
Male, n (%)	17 (100.0)	1988 (93.2)	0.6236
Age (mean±SD), years	28.0 (±6.21)	29.0 (±6.5)	0.5109
Risk, n (%)			
MSM	13 (76.5)	1545 (72.4)	0.766
Heterosexual	4 (23.5)	587 (27.5)	
Reasons for screening, n (%)			
HIV-positive sexual partners	4 (23.5)	195 (9.1)	0.0649
History of STI	2 (11.8)	318 (14.9)	0.7168
Having sex-for-money or money-for-sex	2 (11.8)	153 (7.2)	0.3498
Having one-night stands	4 (23.5)	568 (26.6)	0.7733
Having anal sex	11 (64.7)	1109 (52.3)	0.3081
Having unsafe anal sex (without 100% condom use)	5 (33.3)	531 (25.4)	0.7329
Having oral sex	12 (70.6)	1444 (68.1)	0.823
Recreational drug use	2 (11.8)	148 (6.9)	0.4366
Laboratory test results, n (%)			
HIV seroconversion during follow-up	3 (17.7)	132 (6.2)	0.0863
RPR titre of≥4	2 (11.8)	41 (1.9)	0.0439
Recent syphilis	2 (11.8)	82 (3.8)	0.1406

HCV, hepatitis C virus; MSM, men who have sex with men; RPR, rapid plasma reagin; STI, sexually transmitted infection.

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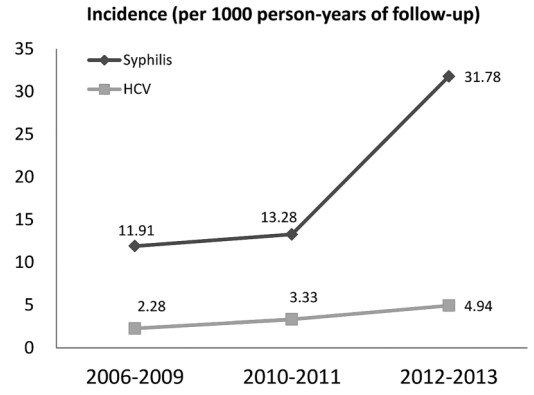


Figure 1 The incidence rates of hepatitis C virus (HCV) and syphilis, 2006–2013.

IDUs, which increased from 2.28 per 1000 PYFU in 2006–2009 to 4.94 per 1000 PYFU in 2012–2013. Having HIV-positive partners was a consistently independent factor associated with HCV seroconversion in two multivariate analyses. Furthermore, the phylogenetic analysis corroborated these results and showed that sequences from HCV seroconverters among VCT clients tended to cluster with those from HIV-positive MSM.

Similar to the findings observed in several Western countries, the increasing trends of HCV infection in HIV-positive MSM have also been found in East Asia, in regions of Japan,[28] Hong Kong[29] and Taiwan,[18] in recent years. However, few studies addressed the incidence rates of HCV infection for transmission of STIs among the HIV-negative populations who engaged in high-risk behaviours. In the van de Laar *et al*[13] study, there were no HCV seroconverters among HIV-negative MSM during two study periods (1984–1999 and 2000–2003). In contrast, Richardson *et al*[15] reported that the incidence of HCV infection in HIV-negative MSM increased from 0 per 1000 PY during 2000–2003, 0.7 per 1000 PY in 2004 and 1.5 per 1000 PY in 2005, to 5.8 per 1000 PY in 2006. Such discrepancies might result from a lack of data from HIV-negative MSM in the former study because regular HCV screening was not recommended for MSM without HIV infection when the study was conducted. In our study, the increasing incidence of HCV infection in HIV-negative MSM concurs with the findings of Richardson *et al*. Such findings would support the counselling at VCT visit that provides information, communication and education about traditional STIs, such as syphilis, gonorrhoea and chlamydia, as well as viral hepatitis.

In the two analyses, we found that having HIV-positive partners was consistently an independent factor associated with HCV seroconversion, while other risk factors, such as history of STIs, anal sex, one-night stands, unsafe anal sex (defined as no 100% condom use), oral sex and use of non-injection drugs, were not statistically significantly different between HCV seroconverters and non-seroconverters. HIV infection may increase both infectiousness and susceptibility to HCV. In the study by Sherman *et al*,[30] HIV increased serum HCV RNA load by more than 1 $\log_{10}$ IU/mL in HCV/HIV-co-infected individuals compared to those with HCV-mono-infection, and HCV/HIV-co-infected patients were more likely to shed HCV RNA in semen compared with HCV-mono-infected patients, suggesting a possible role in sexual transmission.[31] Although we did not have HCV data from those HIV-positive partners, the finding of clustering of HCV sequences in phylogenetic analysis between HIV-positive and HIV-negative MSM in our study suggests such a link of HCV transmission.

Our study found that an RPR titre of four or greater was associated with HCV seroconversion in multivariate analysis (table 2). Ulcerative STIs, such as syphilis or lymphogranuloma venereum (LGV), could facilitate HCV acquisition from disrupted mucosa. In a case–control study,[22] individuals with a history of syphilis, gonorrhoea or chlamydia in the past 12 months were associated with HCV seroconversion in univariate analysis (OR 2.78, 95% CI 1.18 to 6.5). In the study by Sun *et al*,[18] in Taiwan, HIV-positive MSM with recent syphilis had an approximately 7.3-fold higher risk of HCV seroconversion than those without recent syphilis. Our finding of concurrently increasing trends of syphilis lends support to the association between syphilis and HCV seroconversion among our VCT clients (figure 1).

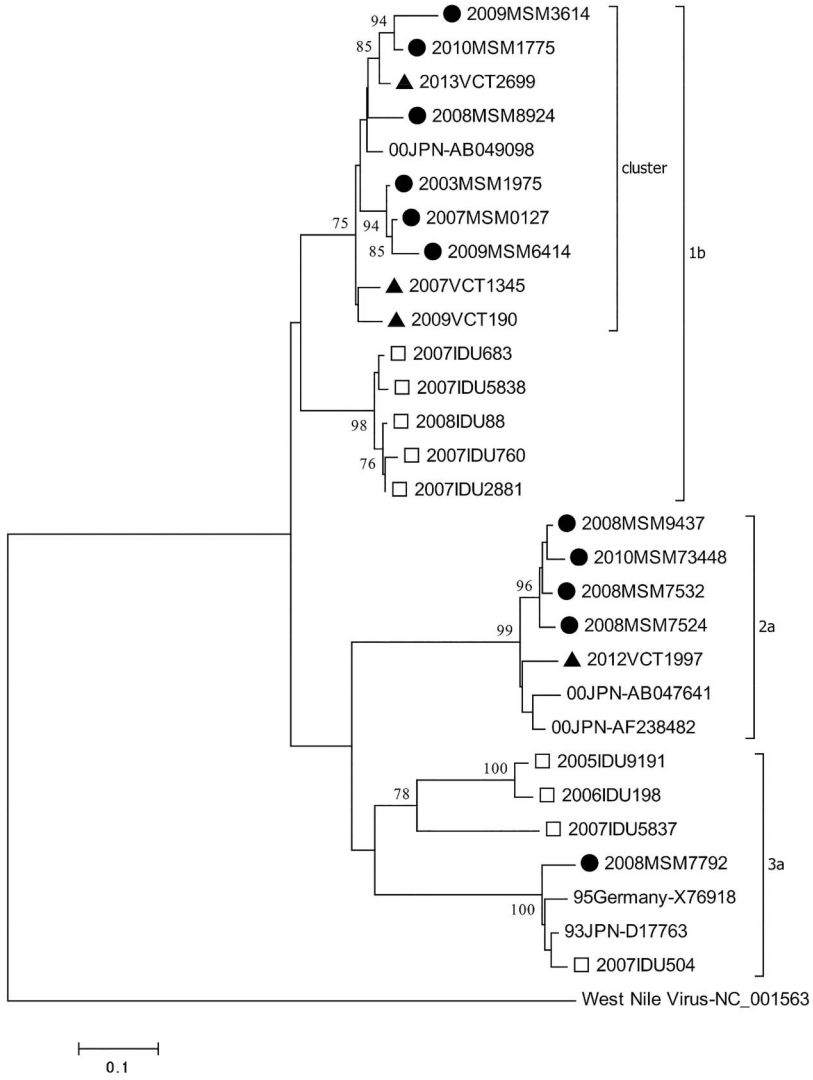
Changes of risk behaviours may also be an important issue in sexual transmission of HCV infection. In London, a large survey of changes in sexual behaviours of MSM was performed between 1998 and 2008.[32] The percentage of MSM who had unprotected anal intercourse (UAI) increased from 24.3% in 1998 to 36.6% in 2008. In another UK study,[33] 60 cases and 130 controls showed that UAI, rimming, fisting, use of sex toys and group sex

Table 2 Multivariate analysis of factors associated with HCV seroconversion among all clients

Variables	Reference	AHR (95% CI)	p Value
Age, years	Continuous variable	0.927 (0.843 to 1.02)	0.1209
HIV-positive sexual partners	Unknown or HIV-negative sexual partners	3.756 (1.18 to 11.955)	0.0251
MSM	Heterosexual	0.852 (0.267 to 2.761)	0.7867
Use of recreational drugs	No use of recreational drugs	1.252 (0.241 to 6.5)	0.7889
History of STI	No history of STI	0.332 (0.061 to 1.811)	0.2031
RPR titre of ≥4	RPR titre of <4	9.978 (1.55 to 64.233)	0.0155

AHR, adjusted HR; HCV, hepatitis C virus; MSM, men who have sex with men; RPR, rapid plasma reagin; STI, sexually transmitted infection.

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(●: MSM infected with HIV and HCV ; ▲: VCT clients; □: IDU infected with HIV and HCV.)

Figure 2 Phylogenetic analysis of NS5B sequences amplified from HCV strains from HCV seroconverters (closed triangles), HIV-positive MSM (closed circles) and HIV-positive injecting drug users (open squares). The GenBank accession numbers of the obtained partial NS5B HCV sequences used in the phylogenetic analysis were JQ060117–121, JQ060123–124, JQ060126, JQ060131–133, HM031199, HM031203–204, HM031211, DQ179117, DQ663604, DQ663608, DQ666268 and EU255966 (HCV, hepatitis C virus; IDU, injecting drug user; MSM, men who have sex with men; VCT, voluntary counselling and testing).

participation were associated with HCV acquisition in univariate analysis; moreover, sex in a group (more than two individuals) was the strongest predictor for HCV acquisition in this study. Similarly, Schmidt *et al*[22] identified rectal trauma with bleeding, receptive fisting without gloves (or with shared gloves), group sex and nasally administered drugs were significant risks. The authors emphasised that rectal bleeding caused by fisting or prolonged anal intercourse leads to lesions in anal mucosa, which could serve as a portal of entry and facilitate HCV transmission. However, our questionnaire, in addition to querying on condom use, did not include inquiries about risk behaviours that may cause bleeding.[25] Thus, we were not able to assess if those risky behaviours were associated with HCV seroconversion in our study.

There are several limitations in our study. First, we used a questionnaire to collect information on sexual risk behaviours. It is difficult to avoid recall bias of VCT clients and all data were self-reported; therefore we were not able to verify the information provided. Second, we did not perform HCV RNA for all clients who were HCV-seronegative. This could underestimate the incidence rate of HCV infection. Third, the number of HCV seroconversion cases remains small, which may preclude us from identifying other associated factors with HCV seroconversion. Fourth, our study was conducted at a single VCT site in Taiwan. More surveillance studies are warranted to confirm our findings of an increasing incidence rate of HCV infection in HIV-negative populations engaged in risk behaviours for STIs in Taiwan and other countries in the Asia-Pacific region.

In conclusion, we demonstrate an increasing trend of recent HCV infection among individuals who sought VCT services in Taiwan. Syphilis and having HIV-positive partners are two factors that are independently associated with recent HCV seroconversion.

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Contributors C-CH contributed to the study conception and design. S-YC, Y-CS and C-HW carried out the laboratory work. P-YW, Y-ZL and L-HC collected the demographic data. W-CL and J-CT performed the statistical analysis. J-CT, who interpreted the data and wrote the draft, was the lead author. C-CH, H-YS and S-CC supervised and revised the work critically for intellectual content. All the authors contributed to this manuscript.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval This study was approved by the Research Ethic Committee of National Taiwan University Hospital.

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Increasing incidence of recent hepatitis C virus infection among persons seeking voluntary counselling and testing for HIV and sexually transmitted infections in Taiwan

Jen-Chih Tsai, Chien-Ching Hung, Sui-Yuan Chang, Wen-Chun Liu, Cheng-Hsin Wu, Yi-Ching Su, Pei-Ying Wu, Yu-Zhen Luo, Lan-Hsin Chang, Hsin-Yun Sun and Shan-Chwen Chang

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RESEARCH ARTICLE

Presence of Tablet Remnants of Nevirapine Extended-Release in Stools and Its Impact on Virological Outcome in HIV-1-Infected Patients: A Prospective Cohort Study

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Abstract

Background

Nevirapine extended-release (NVP-XR) taken once daily remains an effective antiretroviral agent for patients infected with HIV-1 strains that do not harbor resistance mutations. Presence of tablet remnants of NVP XR in stools was reported in 1.19% and 3.05% of subjects in two clinical trials. However, the prevalence may have been underestimated because the information was retrospectively collected in the studies.

Methods

Between April and December 2014, we prospectively inquired about the frequency of noticing tablet remnants of NVP XR in stools in HIV-1-infected patients who switched to antiretroviral regimens containing NVP XR plus 2 nucleos(*t*)ide reverse-transcriptase inhibitors. Patients were invited to participate in therapeutic drug monitoring of plasma concentrations of NVP 12 or 24 hours after taking the previous dose (C12 and C24, respectively) of NVP XR using high-performance liquid chromatography. The information on clinical characteristics, including plasma HIV RNA load and CD4 lymphocyte count, at baseline and during follow-up was recorded.

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Competing Interests: C-CH has received research support from Janssen and speaker honoraria from Abbvie, Bristol-Myers Squibb, and Gilead Sciences, and served on advisory boards for Gilead Sciences and Abbvie. The research support from Janssen was not related to the current study. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials. Other authors have no competing interests to declare.

Results

During the 9-month study period, 272 patients switched to NVP XR-based regimens and 60 (22.1%) noticed tablet remnants of NVP XR in stools, in whom 54.2% reported noticing the tablet remnants at least once weekly. Compared with patients who did not notice tablet remnants, those who noticed tablet remnants had a higher mean CD4 lymphocyte count (629 vs 495 cells/mm^3, $P = 0.0002$) and a similar mean plasma HIV RNA load (1.57 vs 1.61 $\log_{10}$ copies/mL, $P = 0.76$) on switch. At about 12 and 24 weeks after switch, patients who noticed tablet remnants continued to have a similar mean plasma HIV RNA load (1.39 vs 1.43 $\log_{10}$ copies/mL, $P = 0.43$; and 1.30 vs 1.37 $\log_{10}$ copies/mL, $P = 0.26$, respectively), but had a lower median NVP C12 (3640 vs 4730 ng/mL, $P = 0.06$), and a similar median NVP C24 (3220 vs 3330 ng/ml, $P = 0.95$) when compared with those who did not notice tablet remnants.

Conclusions

The presence of tablet remnants of NVP XR in stools is not uncommon in HIV-1-infected Taiwanese patients receiving NVP XR-based antiretroviral regimens, which does not have an adverse impact on the virological and immunological outcomes.

Introduction

Nevirapine (NVP), a potent non-nucleoside reverse-transcriptase inhibitor (nNRTI), has been an important component of antiretroviral therapy for HIV-1 infection with high efficacy and lower rates of metabolic complications over the past two decades, although the adverse effects such as hepatotoxicity and hypersensitivity have precluded it from the preferred antiretroviral regimens [1–3]. The 2NN study demonstrated comparable virological response between patients treated with NVP immediate-release [NVP IR]) 200 mg twice daily and those treated with NVP IR 400 mg once daily [2], and both groups of subjects had similar exposure to NVP as indicated by 24 hours area-under-the-curve (AUC_{24h}) [4].

To reduce the pill burden and enhance convenience and adherence, NVP IR was replaced by NVP extended-release (NVP XR) tablet that contains 400 mg of NVP in a single tablet to be taken once daily. The NVP XR tablet contains hydrophilic polymer hydroxypropyl methylcellulose, which provides extended, controlled release of NVP in the gastrointestinal tract. Although the NVP XR formulation had a lower bioavailability than NVP IR, trough plasma concentration with NVP XR was similar to that of NVP IR and no virological failure was reported in patients treated with NVP XR formulations [5].

In two clinical trials (VERxVE and TRANxITION), the NVP XR formulation has demonstrated non-inferiority of efficacy and similar safety to NVP IR formulation, although the exposure to NVP and the peak NVP concentration are lower in patients taking NVP XR than those taking NVP IR [6,7]. Presence of tablet remnants of NVP XR in stools has been reported to occur in 1.19% and 3.05% of the subjects in the two trials, respectively, by retrospective investigation [8]. However, such a prevalence may have been underestimated due to the fact that the information was obtained by self-reporting. Another post-marketing population-based cohort study with NVP XR tablets reported that 31 out of 536 (6%) patients had "whole" tablet in their stools, which was not associated with adverse outcomes [9].



Fig 1. A photograph taken by one of the patients showing a washed tablet remnant of nevirapine extended-release collected from stools.

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In this prospective cohort study, we aimed to determine the prevalence of tablet remnants of NVP XR in stools and to evaluate its impact on virological and immunological outcomes in HIV-1-infected patients who switched to NVP XR-based antiretroviral regimens. Therapeutic drug monitoring of plasma NVP concentrations was performed in a subgroup of patients switching to NVP XR-based regimens.

Methods

Patients

From April to December, 2014, we enrolled HIV-1-infected patients who switched to NVP XR plus 2 nucleos(*t*)ide reverse-transcriptase inhibitors. These patients were interviewed with the use of a face-to-face questionnaire to inquire about the frequency of noticing tablet remnants of NVP XR in stools (Fig 1) when the patients returned for the clinic appointments. Because

the patients rarely paid attention to the contents of the stools, they were instructed to see if any tablet remnants were present in stools within the next 4 weeks after clinic visits and our case managers made cell phone calls to inquire about the results.

We used a computerized data collection form to record clinical characteristics that included age, gender, risk of HIV-1 transmission, co-infection of hepatitis B virus (HBV) or hepatitis C virus (HCV), frequency of noticing NVP XR remnants in stools, backbone antiretroviral therapy, duration of NVP XR before the study, concurrent medications, plasma HIV RNA load and CD4 lymphocyte count on and after switch to NVP XR-based regimens. Patients were invited to participate in therapeutic drug monitoring of plasma NVP concentrations with the use of high-performance of liquid chromatography (HPLC) 12 or 24 hours after taking the previous dose. Clinical characteristics, plasma NVP concentrations, and immunological and virological outcomes were compared between patients with and those without noticing tablets remnants of NVP XR in stools. The study was approved by the Research Ethics Committee of the hospital (registration numbers, 201103077RC and 20103112R) and the patients gave written informed consent before participating in the study.

Laboratory investigations

After switch to NVP XR-based regimens, plasma HIV RNA load and CD4 lymphocyte count were followed every 3 to 6 months. CD4 lymphocyte count was determined using flow cytometry (BD FACS Calibur, Becton Dickinson and Coulter Epics XL, Beckman Coulter, CA, USA). Plasma HIV RNA load was quantified using the Cobas AmpliPrep/Cobas TaqMan HIV-I test (version 2.0, Roche Molecular Systems, Inc.) with a lower detection limit of 20 copies/mL.

Since 2009, we started to conduct therapeutic drug monitoring of plasma concentrations of efavirenz, nevirapine and atazanavir among HIV-infected patients [10]. Patients' blood samples were collected into potassium and ethylenediaminetetraacetic-acid–containing 10-mL tubes. They were centrifuged and plasma was stored at -20°C prior to analysis. The plasma concentration of NVP was analyzed using HPLC with a modified method reported by van Heeswijk [11]. In brief, 300 μL of the plasma were mixed with 300 μL of acetonitrile for 30 seconds. The tubes were then centrifuged for 10 minutes at 10500 g, and 300 μL of the clear supernatant was evaporated to dryness under a gentle stream of nitrogen at 30°C. The residues were then dissolved in 200 μL of mobile phase, mixed for 30 seconds and centrifuged for 3 minutes at 10500 g. The clear supernatants were transferred to HPLC autosampler vials with inserts. The HPLC system consisted of a L-2130 HTA solvent delivery pump, a L-2200 autosampler, a L-2420 UV-Vis detector, and the HPLC D-2000 Elite on Windows (version 1.2, Hitachi High Technologies Corporation, Tokyo, Japan) chromatographic data system. Chromatography was performed on a Mightysil RP-18 GP column (250 x 4.6 nm, 5 μm; Kanto Corporation, Portland, OR). The mobile phase was composed of the phosphate buffer (pH = 4.0, 10 mM) mixed with acetonitrile at a ratio of 70:30 (vol/vol), and the flow rate was 1 mL/min. The detection wavelength was at 250 nm. The retention time of NEV was 6.31 minutes. The calibration curve of NVP was linear over the range 100 to 10000 ng/mL ($y = 54403X+5045.3$, $R = 0.9997$). The lower limit of quantification was 100 ng/mL. The extraction recovery was 97.22%. The accuracy ranged from 95.10 to 102.61%. The peak area of intra-assay and inter-assay coefficients of variation at 5000 ng/mL ranged from 0.09 to 0.58% and 0.12 to 0.63%, respectively.

Statistical analysis

Statistical analyses were performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were reported as mean ± standard deviation, and compare with Student's *t* test. Categorical variables were expressed as percentage of the total number of

Table 1. Comparisons of baseline clinical characteristics of HIV-1-infected patients who noticed and those who did not notice tablet remnants of nevirapine extended-release (NVP-XR) in stools.

Variables	NVP XR remnant n = 60	No remnant n = 212	Statistics *P* value
Age, mean (SD), years	34.7 (8.0)	37.9 (11.9)	0.02
Male sex, n (%)	60 (100.0)	202 (95.3)	0.09
Risk for HIV transmission, n (%)			0.13
MSM/bisexual	58 (96.67)	189 (89.57)	
Heterosexual	1 (1.67)	20 (9.48)	
Others	1 (1.67)	3 (1.11)	
HBsAg-positive, n (%)	4 (6.67)	15 (7.08)	0.91
Anti-HCV-positive	2 (3.33)	17 (8.02)	0.21
Concurrent medications, n (%)			
Anti-hypertensives	2 (3.33)	14 (6.60)	0.34
Anti-diabetes	1 (1.67)	5 (2.36)	0.75
Lipid-lowering agents	1 (1.67)	10 (4.72)	0.29
Anti-HCV therapy	0	0	
PVL on switch to NVP XR, mean (SD), log_{10} copies/ml,	1.57 (0.84)	1.61 (0.94)	0.76
PVL on switch to NVP XR, PVL <50 copies/ml, n (%)	53 (88.33)	180 (86.12)	0.66
CD4 on switch to NVP XR, mean (SD), cells/mm^3	629 (271)	495 (227)	0.0002
Backbone ARV, n (%)			
Abacavir/lamivudine	16 (26.67)	64 (30.19)	0.60
Tenofovir/lamivudine or tenofovir/emtricitabine	38 (63.33)	115 (54.25)	0.21
Zidovudine/lamivudine	4 (6.67)	32 (15.09)	0.09
Others	2 (3.33)	1 (0.47)	0.06
Duration of exposure to NVP XR before enrollment, mean (SD), days,	158 (66)	160 (73)	0.84

Abbreviations: ARV, antiretroviral therapy; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; MSM, men who have sex with men; PVL, plasma HIV-1 RNA load; SD, standard deviation

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patient analyzed, and compare with chi-squared test. Plasma NVP concentrations of patients who noticed tablet remnants and those who did not were expressed by median and interquartile range (IQR) and were compared by Kruskal-Wallis test. A *P* value less than 0.05 was considered statistical significance.

Results

During the 9-month study period, 310 patients who had switched to NVP XR plus 2 nucleos(*t*)ide reverse-transcriptase inhibitors were identified from the Department of Pharmacy of the hospital; 272 patients (87.7%) agreed to participate in the questionnaire interview, in whom 60 patients (22.1%) reported noticing tablet remnants of NVP XR in stools on face-to-face or cell phone questionnaire interview. The baseline clinical characteristics, virological and immunogical outcomes of HIV-1-infected patients with or without tablet remnants of NVP XR in stools are summarized in Table 1. Frequency of noticing NVP XR tablet remnants in stools ranged from once per day to 1 to 2 times every 3 months, with 53.3% of the patients noticing tablet remnants in stools at least once weekly (Table 2).

Compared with the patients who did not notice tablet remnants of NVP XR in stools, those who noticed tablet remnants were younger (34.7 vs 37.9 years, $P = 0.02$), and had similar mean plasma HIV RNA load (1.57 vs 1.61 log_{10} copies/mL, $P = 0.76$), a higher mean CD4 lymphocyte count (629 vs 495 cells/mm^3, $P = 0.0002$) on switch to NVP XR-based regimens, and a

similar duration of exposure to NVP XR before enrollment into this study (mean duration, 158 vs 160 days, $P = 0.84$) (Table 1).

After a mean duration of 100 days of switch to NVP XR in the first routine clinical follow-up, patients noticing tablet remnants had a similar mean plasma HIV RNA load (1.39 vs 1.43 $\log_{10}$ copies/mL, $P = 0.43$), a similar percentage of virological suppression (93.2% vs 92.3%, $P = 0.81$), and a higher mean CD4 lymphocyte count (660 vs 541 cells/mm^3, $P = 0.0008$) compared with those who did not notice tablet remnants. Second clinical follow-up, at a mean duration of 213 days after switch, also demonstrated a similar mean plasma HIV RNA load (1.30 vs 1.37 $\log_{10}$ copies/mL, $P = 0.26$), percentage of virological suppression (<50 copies/ml) (96.0% vs 95.0%, $P = 0.79$), and mean CD4 lymphocyte count (628 vs 560 cells/mm^3, $P = 0.10$) between the patients who noticed and those who did not notice tablet remnants (Table 2).

A total of 89 patients (32.7%) participated in therapeutic drug monitoring of plasma NVP concentrations: 44 patients noticing tablet remnants (including 34 and 10 patients who had determinations of plasma NVP concentrations at 12 and 24 hours of the previous dosing, respectively) and 45 patients not noticing remnants (including 31 and 14 patients who had determinations of plasma NVP concentrations at 12 and 24 hours of the previous dosing, respectively). The clinical characteristics were similar between the two subgroups of patients who underwent therapeutic drug monitoring of plasma NVP concentrations (Table 3). The median plasma NVP concentration at 12 hours of the previous dosing was lower for the 34 patients who noticed tablet remnants in stools than that for the 31 patients who did not notice remnants (3640 ng/mL [IQR 3230–4880] vs 4730 ng/mL [IQR 3340–5910], $P = 0.06$), while the median plasma NVP concentrations at 24 hours of the previous dosing were similar between the two groups (3220 ng/mL [IQR 2580–5070] vs median 3330 ng/mL [IQR 2930–4280], $P = 0.84$) (Fig 2).

Discussion

This is the first prospective cohort study to determine the frequency of noticing tablet remnants of NVP XR in stools and its impact on virological and immunological outcomes in HIV-

Table 2. Frequency of noticing nevirapine extended-release (NVP-XR) tablet remnants and comparisons of virological and immunological outcomes of HIV-1-infected patients with and without noticing tablet remnants of NVP XR in stools.

Variables	NVP XR remnants n = 60	No remnants n = 212	Statistics *P* value
Frequency of NVP XR remnants in stools, n (%)			
Daily	6 (10.0)	NA	
2–5 times weekly	9 (15.0)	NA	
Once weekly	17 (28.3)	NA	
2–3 times monthly	6 (10.0)	NA	
Once monthly	12 (20.0)	NA	
1–2 times over a 3-month period	9 (15.0)	NA	
First PVL after switch to NVP XR, mean ± SD, $\log_{10}$ copies/ml, (n = 59/207)	1.39 (0.30)	1.43 (0.48)	0.43
First PVL < 50 copies/ml, n (%)	55 (93.2)	191 (92.3)	0.81
First CD4 after switch to NVP XR, mean (SD), cells/mm^3, (n = 58/207)	660 (252)	541 (232)	0.0008
Interval between switch and first blood sampling, days, mean (SD)	95.6 (55.2)	101.5 (54.6)	0.47
Second PVL after switch to NVP XR, mean (SD), $\log_{10}$ copies/ml (n = 48/137)	1.3 (0.12)	1.37 (0.37)	0.26
Second PVL <50 copies/ml, n(%)	46 (96.0)	130 (95.0)	0.79
Second CD4 after switch to NVP XR, mean (SD), cells/mm^3 (n = 48/134)	628 (270)	560 (229)	0.10
Interval between switch and second blood sampling, mean (SD), days	213 (72)	214 (58)	0.92

Abbreviations: ARV, antiretroviral therapy; MSM, men who have sex with men; NA, not applicable; PVL, plasma HIV-1 RNA load; SD, standard deviation; C12, plasma concentration 12 hours after the previous dosing; C24, plasma concentration 24 hours after the previous dosing

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Table 3. Comparisons of clinical characteristics and virological and immunological outcomes of HIV-1-infected patients with and without noticing tablet remnants of nevirapine extended-release (NVP-XR) in stools, who underwent therapeutic drug monitoring of plasma concentration of nevirapine.

Variables	NVP XR remnants n = 44	No remnants n = 45	Statistics *P* value
Age, mean (SD), years	34.7 (7.82)	37.1 (11.06)	0.24
Male sex	44 (100.0)	43 (95.56)	0.16
Risk for HIV transmission			0.09
MSM/bisexual, n (%)	42 (95.45)	39 (86.67)	
Heterosexual, n (%)	1 (2.27)	6 (13.33)	
Others, n(%)	1 (2.27)	0 (0.00)	
HBsAg-positive, n (%)	2 (4.55)	1 (2.22)	0.54
Anti-HCV-positive, n (%)	0 (0.00)	2 (4.44)	0.16
Concurrent medications			
Anti-hypertensives	2 (4.55)	4 (8.89)	0.41
Anti-diabetes	1 (2.27)	1 (2.22)	0.99
Lipid-lowering agents	0 (0.00)	2 (4.44)	0.16
Anti-HCV therapy	NA	NA	NA
PVL on switch to NVP XR, mean (SD), log_{10} copies/ml	1.51 (0.66)	1.39(0.29)	0.33
CD4 on switch to NVP XR, mean (SD), cells/mm^3	598.3 (249.8)	495.8 (201.1)	0.07
Backbone ARV, n (%)			
Abacavir/lamivudine	12 (27.3)	9 (20.0)	0.42
Tenofovir/lamivudine or tenofovir/emtricitabine	27 (61.4)	35 (77.8)	0.09
Zidovudine/lamivudine	3 (6.8)	1 (2.2)	0.29
Others	2 (4.6)	0 (0.0)	0.15
Duration of exposure to NVP XR before study, mean (SD), days	150 (65.7)	140 (57.7)	0.45
Frequency of NVP XR remnant in stools			
Daily	4 (9.09)	NA	
2–5 times weekly	9 (20.45)	NA	
Once weekly	12 (27.27)	NA	
2–3 times monthly	4 (9.09)	NA	
Once monthly	7 (15.91)	NA	
1–2 times over 3-month period	8 (18.18)	NA	
First PVL after switch to NVP XR, mean (SD), log_{10} copies/ml (n = 43/45)	1.38 (0.30)	1.41 (0.39)	0.70
First CD4 after switch to NVP XR, mean (SD), cells/mm^3 (n = 42/44)	657 (265)	539 (226)	0.03
Interval between switch and first blood sampling, mean (SD), days	93 (52)	105 (62)	0.31
Second PVL after switch to NVP XR, mean (SD), log_{10} copies/ml (n = 37/30)	1.31 (0.06)	1.34 (0.10)	0.19
Second CD4 after switch to NVP XR, mean (SD), log_{10} copies/ml (n = 37/29)	629 (279)	562 (260)	0.32
Interval between switch and second blood sampling, mean (SD), days	213 (75.5)	212 (52.3)	0.99
C12 of NVP, median (IQR), ng/mL (n = 34/31)	3640 (2330–4880)	4730 (3340–5910)	0.06
C24 of NVP, median (IQR), ng/mL (n = 10/14)	3220 (2570–5070)	3330 (2930–4280)	0.95

Abbreviations: ARV, antiretroviral therapy; MSM, men who have sex with men; NVP XR, nevirapine extended-release; PVL, plasma HIV-1 RNA load; SD, standard deviation; C12, plasma concentration 12 hours after previous dosing; C24, plasma concentration 24 hours after previous dosing; IQR: interquartile range

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1- infected patients. Using a face-to-face and cell phone questionnaire interviews, we found that the prevalence of noticing tablet remnants of NVP XR in stools was significantly higher than those reported in two previous clinical trials (22.1% vs 1.19% and 3.05%, respectively) [6–8]; the prevalences of the two trials may have been underestimated because the information on

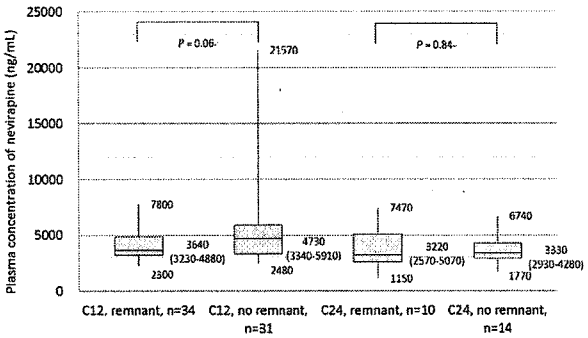


Fig 2. Plasma concentrations of nevirapine 12 hours (C12) and 24 hours (C24) following the previous dosing of nevirapine extended-release in patients noticing and those not noticing tablet remnants in stools. (Numbers in box plots indicate the median, interquartile range, maximum and minimum values).

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noticing tablet remnants of NVP XR in stools was retrospectively obtained by self reporting of the patients. The higher prevalence of noticing tablet remnants of NVP XR in our study may be overestimated due to potential "questionnaire bias", however. The way we obtained the information (face-to-face or cell phone questionnaire interview) might cause the patients to provide anticipated responses. On the contrary, we only performed the questionnaire interview once in a clinical visit or over the next 4 weeks of the visits; the cumulative rate of noticing NVP tablet remnants could be higher.

While the frequencies of tablet remnants of NVP XR in stools differ between our study and the study by Stephan and colleague [8], our findings support the conclusion made by Stephan and colleague that passing NVP XR tablet remnants in stools did not affect the treatment responses to NVP XR-based regimens in the two clinical trials because most of the active ingredient of NVP was absorbed with the residual NVP in the remnants ranging from 22.8 to 44.2% [8]. In our study, we did not observe any adverse impact on the immunological and virological responses to NVP XR-base regimens, either, given the findings that 22.1% of our patients noticed NVP XR remnants in stools and that the median plasma NVP concentration at 12 hours of the previous dosing tended to be lower in the patients with tablet remnants in stools than that in those without (3640 vs 4730 ng/mL, $P = 0.06$).

Therapeutic drug monitoring of NVP concentrations was recommended for efficacy and safety in patient care [12]. At a dose of NVP IR 200 mg twice daily, high exposure to NVP in plasma is associated with an improved virological response [13]. Minimum trough concentration of >3000 ng/mL for NVP had been suggested in a study that included patients with complete as well as those with incomplete adherence [14]. While the target trough concentration for other nevirapine formulations has not been established, Battegay and colleagues found that NVP XR provided a similar trough concentration as NVP IR [5]. In our patients with tablets remnants of NVP XR in stools, who may potentially have sub-therapeutic drug levels, their median trough concentration of NVP remained above the recommended therapeutic drug level (3220 ng/mL). This could explain why tablets remnants of NVP XR in stools had no adverse impact on virological outcomes in our study.

Passage of tablet remnants in stools is not an unusual phenomenon in clinical practices, which may occur whenever patients receive certain pharmaceutical agents that are designed to prolong the rate of drug release [15]. The active compound is continuously released during intestinal passage and its insoluble coats are visibly excreted into stools wholly or in part. The U.S. Food and Drug Administration (FDA) has warned the presence of tablet remnants in

stools of some medications, including NVP XR [16], and declared "this will not affect the way your medicine works". The studies by Stephan and ours should provide sufficient scientific evidence to support the declaration with regard to the impact of NVP XR on virological or immunological outcome.

Our study has several limitations. First, we did not request the patients to bring along the tablet remnants to the clinics for confirmation. Residues of other concurrent medications or undigested materials may have been mistakenly identified as remnants of NVP XR. Second, we were not able to identify the risk factors predisposing to passage of tablet remnants of NVP XR in stools. Passage of tablet remnants of NVP XR may be related to bowel movement, which may have wide day-to-day variation with dietary habits and other factors among individuals. Third, the number of our patients participating in therapeutic drug monitoring of plasma concentrations of NVP remains small and metabolism of nNRTIs may vary with genetic polymorphisms among different ethnicities [10,17,18]. Therefore, our findings among Taiwanese patients may not be generalizable to patients of other ethnicities. Last, we did not measure the residual NVP in the tablet remnants due to limited capacity of the facilities in the laboratory as what Stephan and colleague had done [8].

We conclude that the presence of tablet remnants in stools of NVP XR was not uncommon in HIV-1-infected Taiwanese patients, which did not have an adverse impact on immunological and virological outcome of the patients who switched from NVP IR to NVP XR.

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Author Contributions

Conceived and designed the experiments: Y-CL S-WL S-YC H-YC C-CH. Performed the experiments: S-WL S-YC M-RW H-YC P-YW S-PY W-CL J-YZ Y-ZL Y-CS C-HK. Analyzed the data: S-WL S-YC M-RW P-YW C-CH. Contributed reagents/materials/analysis tools: M-YC W-HS S-MH H-YS C-CH S-CC. Wrote the paper: Y-CL S-WL M-YC S-YC C-CH.

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Transmitted drug resistance of HIV-1 strains among individuals attending voluntary counselling and testing in Taiwan

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Background: Genotypic drug resistance testing for HIV-1 has been integrated into voluntary counselling and testing (VCT) programmes to investigate the trends of transmitted drug resistance (TDR), including integrase mutations, among individuals with recent or chronic HIV infections in Taiwan.

Methods: Between 2006 and 2014, 745 of 21 886 subjects (3.4%) tested HIV positive in the VCT service. The BED assay was used to identify recent HIV infections. Genotypic resistance mutations were interpreted using the WHO 2009 list. Integrase resistance mutations were analysed using the Stanford HIV Drug Resistance Database.

Results: Three-hundred-and-sixty (48.3%) patients were recently infected with HIV-1. Of 440 patients linked to HIV care with analysable reverse transcriptase and protease genes, 49 (11.1%) were infected with HIV-1 harbouring at least one resistance-associated mutation (RAM). The prevalence of TDR to NRTIs, NNRTIs and PIs was 4.1%, 6.4% and 2.3%, respectively. TDR prevalence did not change significantly during the study period. CD4 counts $\leq$500 cells/mm^3 and hepatitis B surface antigen positivity were independent factors associated with acquiring drug-resistant HIV. The prevalence of integrase mutations was 3.2%. Among the seven major integrase mutations (T66I, E92Q, G140S, Y143C/H/R, S147G, Q148H/K/R and N155H), only one strain harbouring the Q148R mutation was detected. We found no statistically significant difference between patients with chronic infection and those with recent infection in the prevalence of drug-resistant mutations to any of the four classes of antiretroviral agents.

Conclusions: The prevalence of TDR of HIV-1 strains to available antiretroviral agents is moderately high, but transmission of HIV-1 with drug-resistant mutations remains stable in Taiwan.

Introduction

The emergence and spread of HIV with drug resistance seems to be inevitable as coverage of ART continues to grow, despite adequate ART options and optimal adherence.[1] This has posed a serious threat to public health globally with the rapid scale-up of ART in low- and middle-income countries, where infrastructures for frequent virological monitoring and testing for drug resistance are lacking.[2]

Transmitted drug resistance (TDR), which occurs when previously uninfected individuals are infected with a drug-resistant HIV strain, is of particular concern because it will jeopardize the success of first-line combination ART (cART).[3–5] The burdens of TDR vary substantially across geographical regions and temporal changes in TDR have been inconsistent in different parts of the world.[6–12] Current data from WHO surveys suggest there is an association between higher levels of ART coverage and a higher prevalence of TDR to NNRTIs.[1] Regular population-based

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surveillance of transmission of drug-resistant strains is critical to ensure sustained effectiveness of ART and to provide the basis for selecting first-line regimens, especially in resource-limited countries where routine HIV drug resistance testing prior to ART initiation is not generally available due to cost constraints.

Conventionally, standard genotypic resistance testing involves testing for mutations in the reverse transcriptase (RT) and protease (PR) genes, and surveillance of TDR is limited to NRTIs, NNRTIs and PIs. However, among the five recommended regimens for ART-naive patients in the recently revised guidelines, four are integrase strand transfer inhibitor (INSTI) based.[13] Currently, transmission of INSTI-resistant virus has rarely been reported among ART-naive patients with HIV infection.[14-16] In addition, integrase resistance mutations may have negative impacts on viral fitness.[17,18] Therefore, routine testing for INSTI resistance is not recommended at present.[13] However, as the use of INSTIs will increase following physician adherence to the guidelines, the potential for transmission of these HIV-1 strains with resistance to INSTIs may also increase. Data on pre-treatment resistance to INSTIs remain scarce among ART-naive patients, especially outside Europe and the USA.[19]

Previous studies on trends of TDR prevalence of HIV-1 in Taiwan showed an increased rate during 2003–06 and a decline during 2007–10, with the use of the HIVdb programme of the Stanford University HIV Drug Resistance Database.[5,20] However, many patients included in prior studies were probably chronically infected, and the prevalence of TDR may be underestimated because drug-resistant strains might revert to WT virus over time in the absence of ART.[21] Anonymous voluntary counselling and testing (VCT) has been demonstrated to reach the target populations most at risk of HIV infection, and patients diagnosed with HIV infection via VCT are more likely to be in an early stage of HIV infection.[22,23] In this study, we evaluated the prevalences of TDR to NRTIs, NNRTIs, PIs and INSTIs among VCT subjects testing positive for HIV, including patients with laboratory-confirmed recent infection.

Methods

Setting of the voluntary counselling and testing programme

The National Taiwan University Hospital (NTUH), the largest hospital providing free-of-charge HIV care in Taiwan, has provided VCT services since 1999, which was expanded in 2006. The number of attendees of the VCT programme at NTUH accounted for ~14% of the total number in Taiwan in recent years. A standardized anonymous, self-administered questionnaire interview (available as Supplementary data at *JAC* Online) designed by the Taiwan CDC has been performed to obtain the information on the demographics, sexual practices, risk behaviours, history of sexually transmitted infections (STIs), number of sexual partners, HIV serostatus of sexual partners, condom use and use of illicit drugs.[22] After interview and counselling by trained counsellors, an 8–10 mL blood sample was collected from each individual for serological tests of HIV infection and other STIs. The results were given to the subjects by mobile phone, using a unique testing code consisting of the first alphabetic character and the last three digits of the national identification card, sex and birth year for identification. Those testing positive would be linked to a clinical care and case management programme that was implemented in 2006. Other details have been described previously.[22] The study was approved by the research ethics committee of the hospital and the patients who were linked to HIV care at the hospital gave written informed consent for determination of TDR and the BED-CEIA (capture enzyme immunoassay).

Setting of HIV care in Taiwan

HIV-infected Taiwanese patients receive HIV care according to the national treatment guidelines at designated hospitals around Taiwan. Medical costs related to HIV care, including cART, quantification of CD4 lymphocyte counts and plasma HIV RNA load (PVL), and management for opportunistic illnesses are totally reimbursed by the Taiwan CDC. Drug resistance testing, however, was not routinely available and restricted to patients experiencing treatment failure and to pregnant women. The Taiwan CDC has implemented nationwide regulations on the use of first-line regimens since March 2011 due to substantial financial pressure in providing HIV care, and NNRTI-based regimens have been the preferred cART. Regimens with higher costs require pre-prescription approval by the Taiwan CDC. Since then, use of NNRTIs has increased markedly. Furthermore, generic versions of antiretroviral agents have been introduced since December 2012.

Determination of recent HIV infections, subtypes and coinfections

Anti-HIV antibody was tested using particle agglutination (SFD HIV 1/2 PA; Bio-Rad FUJIREBIO, Japan) and HIV infection was confirmed using western blotting (MP Diagnostics HIV BLOT 2.2; MP Biomedicals Asia Pacific Pte Ltd, Singapore). Hepatitis B surface antigen (HBsAg), anti-HBs antibody and hepatitis B core antibody (anti-HBc antibody) were determined with the use of enzyme immunoassay (Abbott Laboratories, Abbott Park, IL, USA). Antibodies to hepatitis C virus were determined with the use of a third-generation enzyme immunoassay (Ax SYM HCV III; Abbott Laboratories, North Chicago, IL, USA).

The BED assay was performed in the residual samples. The BED-CEIA (Calypte, MD, USA) determines the proportion of anti-HIV-1-specific IgG in relation to total IgG, based on the observation that the ratio of anti-HIV IgG to total IgG increases with time shortly after HIV infection.[24] If a specimen is reactive in the standard sensitive enzyme immunoassay and has a normalized optical density of <0.8 in the BED assay, the source patient is considered recently infected. The window period of recent infection is 153 days (95% CI 145–166 days). In addition, we also used age <25 years as an indicator of recent infection, according to WHO TDR survey methods, to validate the classification based on the BED assay.[6] The HIV-1 subtypes were determined as described previously.[25]

Determination of drug resistance mutations

For those with confirmed HIV infection and linked to clinical care at NTUH, genotypic resistance assays were performed retrospectively as described previously.[5,20] Resistance-associated mutations (RAMs) were defined by the presence of at least one mutation included in the 2009 WHO surveillance drug resistance mutation list.[26] To determine genotypic resistant mutations related to INSTIs, an 884 bp fragment of integrase coding regions was PCR amplified. The PCR primer pair used was *pol*2950A (5′-TCA KCA CCT GCC ATC TGT TTT CC-3′)/2064A (5′-AYA ARG GRA TTG GAG GAA ATG AAC A-3′). The amplification condition was 35 cycles of 94°C for 15 s, 55°C for 1 min and 72°C for 2 min, and a final extension at 72°C for 7 min. The sequences were submitted to the Stanford HIVdb (http://hivdb.stanford.edu) to determine the mutation pattern and susceptibility.[27] Mutations causing low-level resistance or above were considered clinically relevant.

PVL and CD4 lymphocyte count were quantified by the Cobas Amplicor HIV-1 Monitor™ Test, version 1.5 (Roche Diagnostics Corporation, Indianapolis, USA) and FACSFlow (Becton Dickinson), respectively.

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Statistical analysis

Data were analysed using SAS 9.2 (SAS Institute, NC, USA). Categorical data were analysed using the χ^2 or Fisher's exact test, as appropriate, and continuous variables were compared using the Wilcoxon test. All tests were two-tailed and a P value <0.05 was considered significant.

Results

Characteristics of the study population

From April 2006 to December 2014, 21886 VCT subjects had HIV tests at NTUH (Figure 1). MSM and heterosexuals accounted for 13325 (60.9%) and 8434 (38.5%) of the attendance, respectively. Almost 60% (n=12978; 59.3%) of VCT subjects were aged 20-29 years. Overall, 745 individuals were newly diagnosed with HIV-1 infection, with an overall HIV seroprevalence of 3.4% (95% CI 3.2%-3.7%) (Figure 1). Among the 745 HIV-positive persons, 360 (48.3%) were considered as having recent infections and 376 (50.5%) long-standing infections based on the BED assay. Three-quarters (n=557; 74.8%) of those infected with HIV-1 were linked to HIV care at NTUH, including 264 (47.4%) classified as having recent infections and 286 (51.3%) chronic infections. The characteristics of these two groups of patients are shown in Table 1. In multivariate analysis, age ≤25 years (OR 2.50; 95% CI 1.62-3.86, P<0.001), transactional sex behaviours (OR 2.93; 95% CI 1.23-6.98, P=0.02), oral sex practices (OR 2.14; 95% CI 1.16-3.96, P=0.02) and having CD4 lymphocyte counts >500 cells/mm^3 (OR 1.82; 95% CI 1.16-2.86, P<0.01) and PVL >5 $\log_{10}$ copies/mL (OR 2.41; 95% CI 1.55-3.76, P<0.001) were associated with recent infections.

Approximately 95% of HIV-infected patients were MSM. Overall, 197 (92.9%) of patients with recent infections and 201 (89.7%) of those with chronic infections were infected with HIV-1 subtype B. The second most common subtype was CRF01_AE, which accounted for 3.3% of those with recent infections and 5.8% of those with chronic infections.

TDR to NRTIs, NNRTIs and PIs

Among the 557 HIV-positive patients linked to HIV care at NTUH, 534 (95.9%) had blood specimens available for genotypic resistance tests, and the PR and RT genes were successfully sequenced in 440 of them (82.4%). Patients with analysable RT/PR sequences had significantly higher baseline PVL (4.66 versus 4.46 $\log_{10}$ copies/mL, P=0.02) compared with those without analysable RT/PR sequences, and other baseline demographic and clinical characteristics were generally similar (Table S1, available as Supplementary data at *JAC* Online).

Forty-nine HIV-1 strains (11.1%) harboured at least one PR or RT mutation. The overall prevalence of transmitted RAMs to NRTIs, NNRTIs and PIs was 4.1%, 6.4% and 2.3%, respectively (Figure 1). The prevalence of transmitted RAM to any class of antiretroviral agents (NRTIs, NNRTIs or PIs) was 9.0% and 13.4% (P=0.19) for HIV-1 strains from patients with recent infections and those with

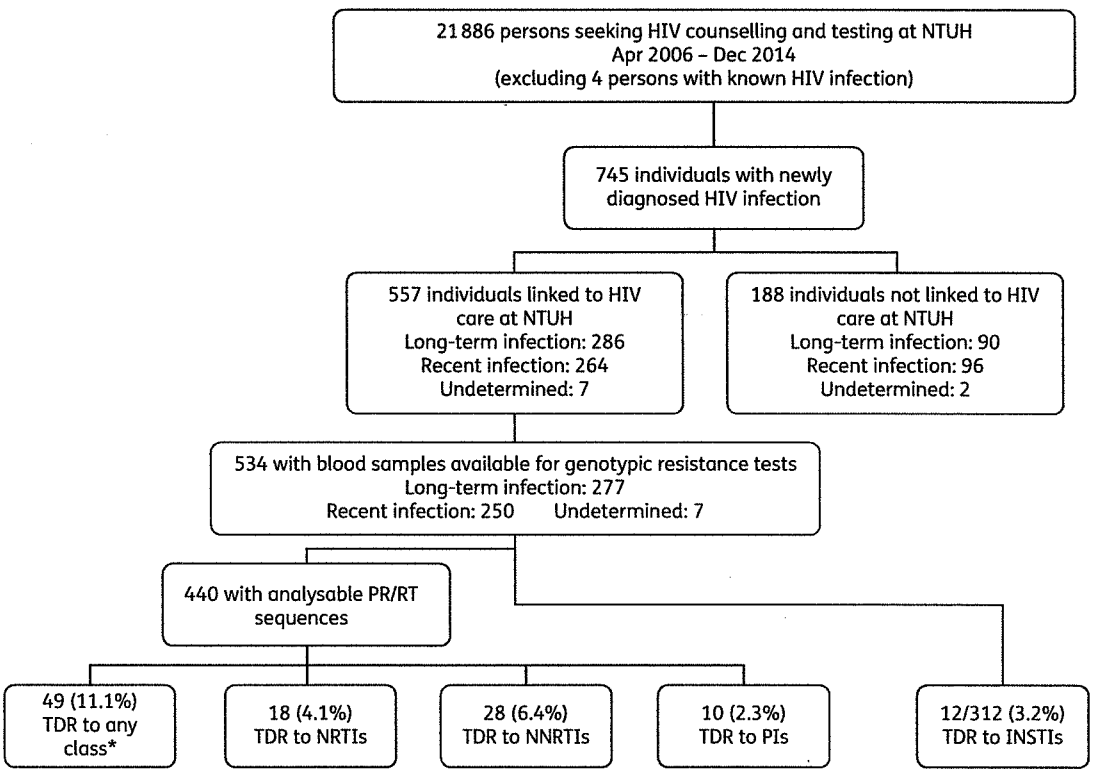


Figure 1. Flow chart of this study. *Any class refers to NRTIs, NNRTIs or PIs.

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Table 1. Comparisons of characteristics between VCT subjects with recent and those with long-term HIV infections who were linked to HIV care

Characteristic	All patients,[a] $N=557$	Recent infections, $N=264$	Long-term infections, $N=286$	P
Male, *n* (%)	543 (97.5)	262 (99.2)	274 (95.8)	0.01*
Age (years), mean ± SD	29.5 ± 7.2	27.5 ± 5.8	31.3 (±7.8)	<0.001*
≤25 years, *n* (%)	163 (29.3)	101 (38.3)	60 (21.0)	<0.001*
Risk, *n* (%)				0.10
MSM	527 (94.6)	255 (96.6)	267 (93.4)	
heterosexual	26 (4.7)	7 (2.7)	18 (6.3)	
IDU with needle sharing	4 (0.7)	2 (0.8)	1 (0.4)	
Reasons for screening, *n* (%)				
having confirmed HIV-infected sexual partners	102 (18.3)	34 (12.9)	67 (23.4)	0.001*
having an IDU partner	2 (0.4)	2 (0.8)	0 (0.0)	0.23
ever having an STI	132 (23.7)	60 (22.7)	71 (24.8)	0.62
having transactional sex	30 (5.4)	21 (8.0)	9 (3.2)	0.01*
having a one-night stand	246 (44.2)	132 (50.0)	110 (38.5)	0.01*
having anal sex	438 (78.6)	231 (87.5)	202 (70.6)	<0.001*
having oral sex	432 (77.6)	225 (85.2)	201 (70.3)	<0.001*
illicit drug use	147 (26.4)	75 (28.4)	70 (24.5)	0.33
Baseline laboratory results				
RPR ≥4, *n* (%)	70 (12.6)	27 (10.2)	42 (14.7)	0.12
western blot for HIV-1, *n* (%)				<0.001*
positive	510 (91.6)	222 (84.1)	283 (99.0)	
indeterminate	37 (6.6)	35 (13.3)	0 (0.0)	
PVL (log_{10} copies/mL), mean ± SD	4.60 ± 0.76	4.75 ± 0.82	4.47 (±0.66)	<0.001*
>5 log_{10} copies/mL, *n* (%)	157 (28.2)	98 (37.1)	56 (19.6)	<0.001*
CD4 cell count (cells/mm^3), mean ± SD	388.4 ± 209.5	436.8 ± 220.7	345.8 (±187.5)	<0.001*
>500 cells/mm^3, *n* (%)	138 (24.8)	83 (31.4)	52 (18.2)	<0.001*
HBsAg positivity, *n* (%)	53 (9.5)	20 (7.6)	32 (11.2)	0.19
anti-HCV positivity, *n* (%)	18 (3.2)	5 (1.9)	11 (3.8)	0.21
available genotypic resistance results, *n* (%)	440 (79.0)	212 (80.3)	224 (78.3)	0.52

HCV, hepatitis C virus; IDU, injecting drug users; RPR, rapid plasma reagin.
*$P<0.05$.
[a]Including seven patients with undetermined duration of HIV-1 infection by the BED assay.

chronic infections, respectively. The prevalence of TDR to each class was not statistically significantly different between patients with recent and those with chronic infections, based on the BED assay (Figure 2a). If we used age <25 years as the only criterion of recent infection, the TDR prevalence of patients with recent and those with chronic infections remained comparable (12.6% versus 10.5%, $P=0.65$) (data not shown).

The overall prevalence of TDR to any antiretroviral agent (NRTIs, NNRTIs or PIs) was 13.9%, 11.5% and 9.8% during 2006–08, 2009–11 and 2012–14, respectively (Figure 2b). The numerically declining trends of TDR to any class of antiretroviral agents did not reach statistical significance. Specific RAMs to NRTIs, NNRTIs and PIs are shown in Figure 3(a). Only K101E/P and K103N/S mutations in RT were observed in >1% of the HIV-1 strains.

Comparisons between patients infected with HIV-1 strains harbouring drug-resistant mutations and those without are shown in Table 2. All HIV strains with transmitted RAMs came from MSM, and all but one were subtype B. CD4 lymphocyte counts ≤500 cells/mm^3 and HBsAg positivity were independent predictors of acquiring drug-resistant HIV in the multivariate model.

TDR-associated mutations to INSTIs

Among 312 HIV-positive patients with genotypic resistance testing of the HIV-1 integrase gene, 216 (69.2%) were enrolled during the period 2012–14. Overall, 10 (3.2%) HIV-1 strains harboured integrase RAMs (Figure 1). The prevalence was 4.3% (1/23) during 2006–08, before the introduction of INSTIs in Taiwan (Figure 2b). The frequencies of individual RAMs are shown in Figure 3(b). If we limited RAMs to major INSTI mutations (T66I, E92Q, G140S, Y143C/H/R, S147G, Q148H/K/R, N155H),[14] only one strain harbouring the Q148R mutation was detected, however.

Discussion

Our study, conducted among VCT subjects testing positive for HIV-1 in Taiwan during 2006–14, showed that 11.1% of the patients were infected with HIV-1 strains harbouring at least

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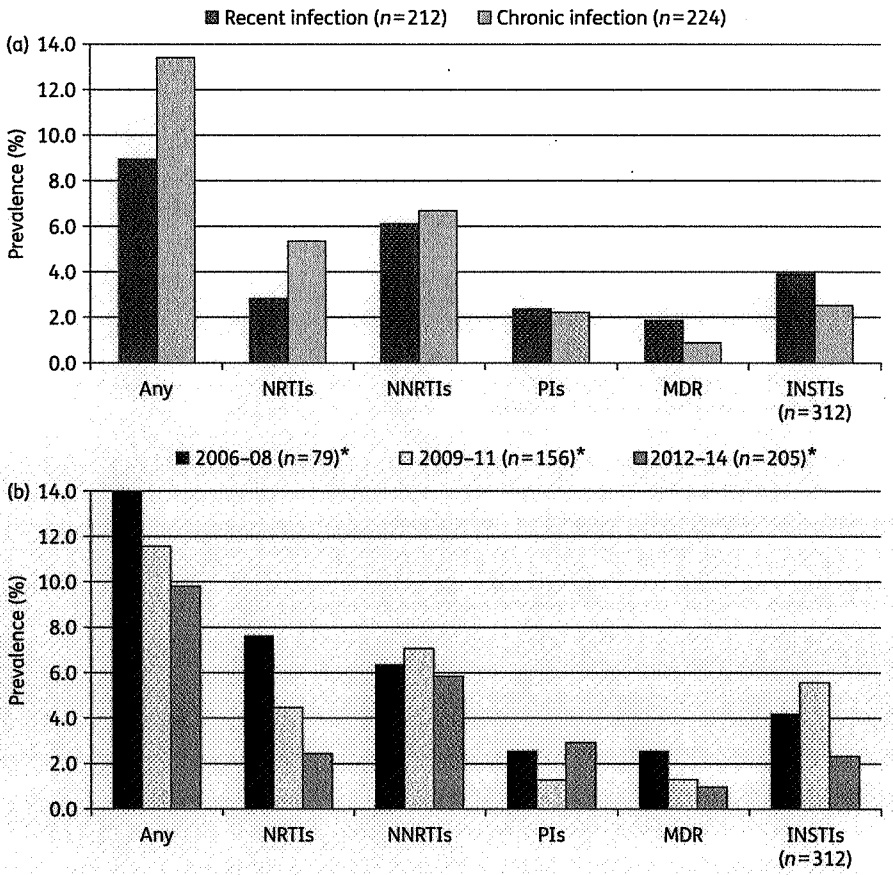


Figure 2. Prevalence of HIV-1 TDR by recent infections (a) and by study periods (b). Any class refers to NRTIs, NNRTIs or PIs. *These numbers did not include genotypic resistance testing of integrase gene.

one PR or RT RAM. Consequently, the prevalence of TDR of HIV-1 in Taiwan was estimated as moderate (5%–15%) according to the WHO categorization method. The prevalence of TDR seemed to be stable throughout the 9 years of the study. We also identified transmission of HIV strains with RAMs to INSTIs for the first time in Taiwan, at a prevalence of 3.2%. In only one patient, a major mutation (Q148R) conferring high-level resistance to two INSTIs in clinical use (raltegravir and elvitegravir) was found.

Our prior surveillance in Taiwan during 2000–10 showed that the overall prevalence of RAMs was 8.0%, and many patients were probably chronically infected due to their low CD4 lymphocyte counts.[5,20] Since transmitted RAMs are more likely to be detected in persons with recent infection compared with those with established infection,[28] it is not surprising that the prevalence in this study seems to be slightly higher. Our HIV-infected patients in the current study were predominately MSM with subtype B, and nearly half of them had recently acquired HIV (within the past 153 days) according to the BED assay. Therefore, this study may be more accurate in estimating the current rate of transmission of RAMs when access to VCT services have been further improved to detect HIV infection early. However, no statistically significant difference was found between chronic and recent infections in the prevalence of one or more drug resistance mutations. In our study, even patients with chronic infections beyond the 153 day window had relatively high CD4 lymphocyte counts (mean 346 cells/mm^3) at baseline, which suggests that the interval between infection and testing was not too extended. Our findings were consistent with a similar surveillance among VCT subjects in southern Taiwan, which reported a 10.6% prevalence of TDR.[29] This prevalence is also in line with those reported in Australia, Europe and North America, in the range of 8%–17%.[8,9,12,23]

To ameliorate the surging costs of antiretroviral drugs, Taiwan CDC, which started to provide free-of-charge ART in the 1990s, has implemented regulations for the prescription of more expensive INSTI- or PI-based regimens. These measures have raised concerns about the emergence of drug resistance, particularly in the absence of routine baseline genotypic resistance testing before cART initiation. In this surveillance, we did not detect any rising trends of TDR to any of the three classes of antiretroviral agents (NRTIs, NNRTIs and PIs). However, mutations conferring resistance to NNRTIs were most common (6.4%), accounting for more than half of the RAMs found. This finding is worrisome, as NNRTI-based regimens are the preferred first-line cART in Taiwan. Vigilant national monitoring in the following years is needed to determine the long-term impacts of the ART policy on the transmission of RAMs and its clinical implications.

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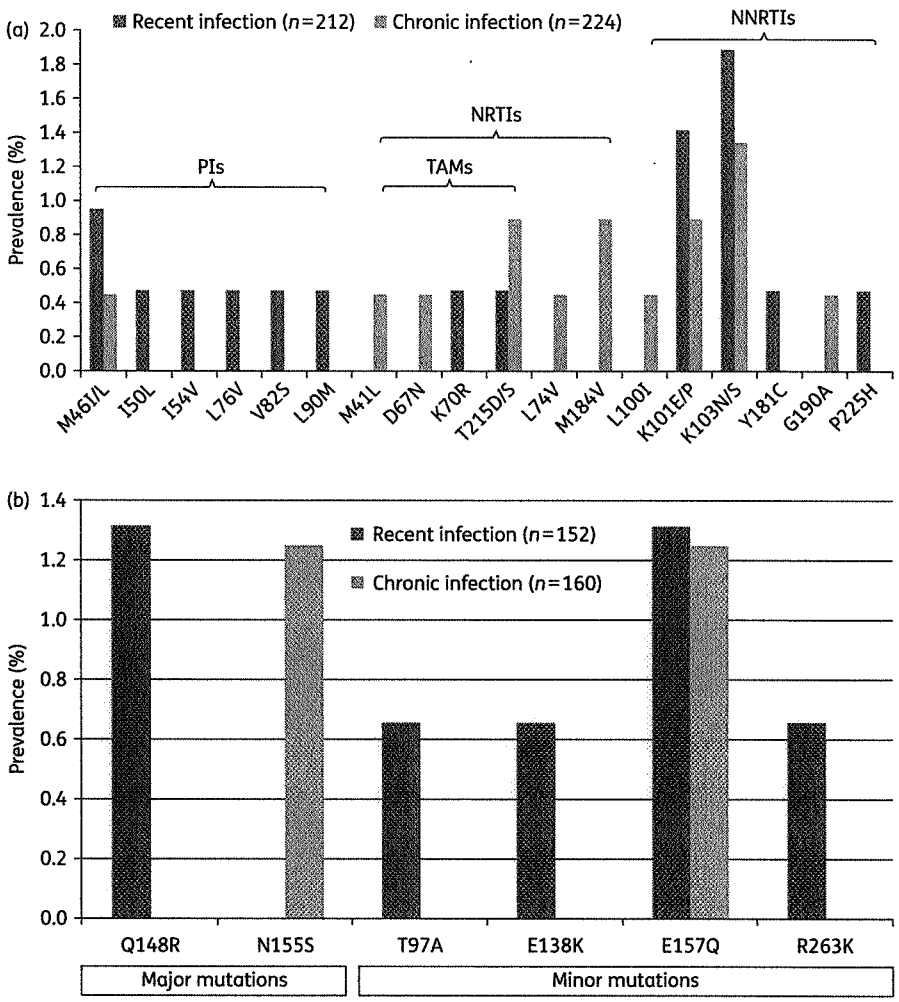


Figure 3. Frequencies of TDR mutations to NRTIs, NNRTIs and PIs (a) and to INSTIs (b). TAM, thymidine analogue mutation.

In this study, CD4 lymphocyte counts ≤500 cells/mm^3 and positive HBsAg were independent predictors of acquisition of HIV with RAMs. To our knowledge, hepatitis B virus (HBV) infection has not been reported to be associated with TDR acquisition. Many of our patients were born after 1986, when universal neonatal HBV vaccination and catch-up vaccination were implemented in Taiwan.[30] In the era of nationwide HBV vaccination for newborns, HBsAg positivity has also been identified to be significantly associated with syphilis and hepatitis C virus antibody (anti-hepatitis C virus) positivity, which implies that HBV might still be transmitted through high-risk sexual behaviours when immunity against HBV wanes and booster vaccination may not be administered.[30] An outbreak of HBV infection with transmission of drug resistance via sexual contacts among HIV-infected patients has also been reported.[31] One possibility of our finding is that drug-resistant HIV might be co-transmitted with HBV through high-risk behaviours. However, this point may not be supported due to similarly high-risk behaviours between patients with and without TDR. Another explanation is HBV/HIV-coinfected patients might be exposed to lamivudine as HBV treatment before HIV diagnosis. Indeed, in our study, two HBsAg-positive patients had received lamivudine monotherapy for HBV before, with their HIV-1 strains harbouring the M184V mutation (data not shown). However, among 18 patients acquiring NRTI-resistant strains, the frequencies of the M184V mutation in HBsAg-positive and HBsAg-negative patients were not significantly different [2/5 (40%) versus 3/13 (23.1%), $P=0.53$] (data not shown). Further studies are needed to elucidate the correlation of HBV coinfection and TDR.

INSTI resistance is potentially influenced by exposure to antiretroviral drugs and HIV-1 subtypes. In clinical trials of ART-naive patients, rates of virological failure with INSTI resistance were low, ranging from 0% to 3%.[17] Raltegravir has been licensed in Taiwan since 2009 and remains the only INSTI in the Taiwanese market. Despite its established efficacy and safety, raltegravir is reserved for patients for whom NNRTI-based regimens are not indicated because of tolerance or resistance issues according to the Taiwanese regulations on cART. In this setting, a prevalence of 3.2% RAMs in integrase, though low, is a point

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Table 2. Comparison of characteristics between patients with resistant strains and those without RAMs

Characteristic	With RAMs, $N=49$	Without RAMs, $N=391$	P	Multivariate analysis	
				OR (95% CI)	P
Year of sampling, *n* (%)			0.59		
2006–08	11 (22.5)	68 (17.4)		1	
2009–11	18 (36.7)	138 (35.3)		1.04 (0.39–2.76)	0.94
2012–14	20 (40.8)	185 (47.3)		0.88 (0.33–2.33)	0.80
Male, *n* (%)	49 (100.0)	380 (97.2)	0.62		0.99
Age (years), mean ± SD	29.2 ± 6.3	29.5 ± 7.1	0.75		
≤25 years, *n* (%)	16 (32.7)	111 (28.4)	0.62	1.65 (0.77–3.57)	0.20
MSM, *n* (%)	49 (100.0)	366 (93.6)	0.10		0.98
Recent HIV infections, *n* (%)	19 (38.8)	193 (49.4)	0.17	0.64 (0.31–1.31)	0.22
Reasons for screening, *n* (%)					
having confirmed HIV-infected sexual partners	9 (18.4)	69 (17.7)	0.84		
having an IDU partner	0 (0.0)	2 (0.5)	>0.99		
ever having an STI	8 (16.3)	97 (24.8)	0.22		
having transactional sex	1 (2.0)	23 (5.9)	0.50		
having a one-night stand	21 (42.9)	176 (45.0)	0.88		
having anal sex	38 (77.6)	312 (79.8)	0.71		
having oral sex	38 (77.6)	312 (79.8)	0.71		
having unsafe oral sex once[a]	33 (86.8)	300 (96.2)	0.05	0.22 (0.02–2.62)	0.23
illicit drug use	9 (18.4)	107 (27.4)	0.23		
Baseline laboratory results					
RPR ≥4, *n* (%)	8 (16.3)	51 (13.0)	0.51		
western blot for HIV-1, *n* (%)			0.23		
positive	46 (93.9)	356 (91.0)			
indeterminate	1 (2.0)	29 (7.4)			
PVL (log_{10} copies/mL), mean ± SD	4.52 ± 0.68	4.68 ± 0.74	0.17		
>5 log_{10} copies/mL, *n* (%)	12 (24.5)	120 (30.7)	0.41	0.62 (0.28–1.37)	0.23
CD4 cell count (cells/mm^3), mean ± SD	336.9 ± 173.5	387.6 ± 201.7	0.09		
>500 cells/mm^3, *n* (%)	5 (10.2)	97 (24.8)	0.02*	0.33 (0.11–0.98)	0.05*
HBsAg positivity, *n* (%)	10 (20.4)	29 (7.4)	0.01*	4.82 (1.94–11.9)	<0.001*
anti-HCV positivity, *n* (%)	0 (0.0)	14 (3.6)	0.38		
subtype B	48 (98.0)	354 (90.5)	0.10	3.08 (0.39–24.11)	0.28

HCV, hepatitis C virus; IDU, injecting drug user; RPR, rapid plasma reagin.
*$P<0.05$.
[a]The denominators were patients having oral sex for each category.

of concern. Among the INSTI-related major mutations we detected, Q148R can cause 30- to 100-fold reduction of drug susceptibility,[32] while N155S is a rare non-polymorphic mutation which was selected *in vitro* and can reduce raltegravir efficacy less efficiently than N155H.[33] Among the four minor mutations detected in this study, T97A and E157Q are polymorphic accessory mutations that can be selected in patients receiving raltegravir,[34] while E138K and R263K are non-polymorphic INSTI-resistance mutations selected in patients receiving INSTIs.[35] At present, none of these minor mutations occurs in combination with INSTI-related major mutations in our study subjects. Further investigations are needed to determine to what extent these RAMs impact the clinical effectiveness of INSTIs. Moreover, additional research is required to determine the source of these mutations. Continuous monitoring is warranted to prevent the spread of strains resistant to INSTIs in the community.

Some questions about INSTI resistance remain unanswered, however. Major INSTI resistance mutations listed in the 2014 edition of the IAS-USA panel and the Stanford University HIV Drug Resistance database are different.[36] Some minor integrase mutations do not reduce susceptibility to INSTIs on their own, but require the presence of other mutations, and minor RAMs have been observed in up to 22.5% of INSTI-naive patients.[27]

The findings of this study should be interpreted with the necessary caution. First, the study was based on data from persons seeking VCT at only one hospital, although this site provides the largest VCT programme and HIV care in Taiwan. Second, the number of cases included in this study was small. Third, our

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participants were predominately MSM infected with subtype B. While MSM account for >85% of HIV infections reported to Taiwan CDC annually after 2008, when the HIV outbreak among injecting drug users was brought under control, this sampling bias precludes generalization of our findings to injecting drug users and heterosexuals. Furthermore, it has been recognized that TDR is most prevalent among MSM worldwide.[12] Thus, TDR epidemics in Taiwan in at-risk populations other than MSM or in subtypes other than B remain to be investigated.

In conclusion, the prevalence of TDR-associated mutations among persons seeking VCT services in Taiwan remains stable and is comparable to the range found in high-income countries. Consequently, baseline genotypic resistance testing should be considered for routine use in newly diagnosed HIV-infected individuals according to the treatment guidelines.[13]

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Author contributions

Conceived and designed the experiments: C.-C. L., W.-C. L., C.-T. F., J.-Y. Y., C.-C. H. and S.-Y. C. Performed the experiments: W.-C. L., J.-Y. Y., L.-H. C., P.-Y. W., Y.-Z. L., S.-F. C., Y.-C. S. and S.-Y. C. Analysed the data: C.-C. L., W.-C. L., C.-T. F., J.-Y. Y., C.-C. H. and S.-Y. C. Wrote the paper: C.-C. L., W.-C. L., C.-C. H. and S.-Y. C.

Supplementary data

The standardized anonymous, self-administered questionnaire interview and Table S1 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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ORIGINAL ARTICLE

Risk of recurrent nontyphoid *Salmonella* bacteremia in human immunodeficiency virus-infected patients with short-term secondary prophylaxis in the era of combination antiretroviral therapy

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Abstract *Background/Purpose:* Nontyphoid *Salmonella* (NTS) bacteremia causes high mortality and recurrence rates in human immunodeficiency virus (HIV)-infected patients. This study aimed to investigate the risk of recurrent NTS bacteremia in the era of combination antiretroviral therapy (cART).
Methods: The medical records of consecutive HIV-infected patients with NTS bacteremia from January 2006 to June 2014 were reviewed. The patients were divided into two groups: patients

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trimethoprim-sulfamethoxazole

who achieved a decline of plasma HIV RNA load by ≥ 2 $\log_{10}$ after 4 weeks of cART (good short-term virological response) and those who failed to achieve the goal (poor short-term virological response). Clinical information was collected on the demographics, immunological and virological responses, prophylactic antibiotics used, episodes of recurrent NTS bacteremia, and mortality.

Results: During the study period, 49 patients with 52 episodes of NTS bacteremia were included: 29 patients in the good virological response group, in which 16 received secondary prophylaxis; and 20 patients in the poor response group, in which 15 received secondary prophylaxis. There were no recurrent episodes of NTS bacteremia in the good-response group, whereas the incidence rate of recurrent NTS bacteremia was 5.21 per 100 person-years and 56.42 per 100 person-years of follow-up in patients receiving and not receiving prophylaxis, respectively, in the poor-response group. No patients died in the good-response group, whereas five patients (25%) in the poor-response group died. The resistance rate of 52 NTS isolates tested to ciprofloxacin was 7.7%.

Conclusion: The risk of recurrent NTS bacteremia is low in HIV-infected patients who achieve short-term virological response to cART, regardless of secondary prophylaxis.

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Introduction

Bacteremia has been an important cause of morbidity and mortality in human immunodeficiency virus (HIV)-infected patients.[1–4] In developing countries, nontyphoid *Salmonella* (NTS) continues to be the leading cause of bacteremia that causes high mortality and recurrence rates.[4] According to the United States Centers for Disease Control and Prevention, recurrent NTS bacteremia is one of the AIDS-defining opportunistic infections.[5] After the introduction of combination antiretroviral therapy (cART), the incidence of bloodstream infections in HIV-infected patients has decreased with the improvement of immunologic status.[6] However, in patients with low CD4 lymphocyte counts, bacteremia, especially NTS bacteremia, remains a threat in developing countries.[1,2,4]

The guidelines of the United States Department of Health and Human Services in 2014 recommend that HIV-infected patients with recurrent gastroenteritis or NTS bacteremia, or those with CD4 counts < 200 cells/μL and severe diarrhea receive secondary prophylaxis against recurrent NTS bacteremia.[9] However, the optimal duration of secondary prophylaxis for this purpose has not been well established, in contrast to the recommendations for primary and secondary prophylaxis against *Pneumocystis jirovecii* pneumonia. It is suggested to stop secondary prophylaxis until the resolution of *Salmonella* infection and response to cART with sustained viral suppression and CD4 cell count > 200 cells/μL.[9] However, the prolonged exposure to antibiotics raises several concerns, such as drug toxicity and increasing antibiotic resistance. In our previous study, the incidence of NTS bacteremia has significantly decreased by 96% after the introduction of cART. Moreover, HIV-infected patients who received fluoroquinolones as secondary prophylaxis for ≤ 30 days did not experience a higher incidence of recurrent NTS bacteremia than those who received secondary prophylaxis for > 30 days. However, the effectiveness of secondary prophylaxis with fluoroquinolones may be compromised given the finding that the proportion of NTS isolates resistant to fluoroquinolones has significantly increased over the three study periods.[8]

In this retrospective study, we aimed to reassess the risk of recurrent NTS bacteremia in the cART era. Our hypothesis is that in HIV-infected patients who receive cART with good adherence and a good short-term virological suppression, prolonged secondary prophylaxis for NTS bacteremia may not be needed.

Methods

Study design and inclusion and exclusion criteria

From January 2006 to June 2014, all HIV-infected patients who were aged 18 years or older and received a diagnosis of NTS bacteremia at the National Taiwan University Hospital, Taipei, Taiwan and Far Eastern Memorial Hospital, New Taipei City, were included in this retrospective observational cohort study. A standardized case record form was used to collect clinical and microbiological data. We excluded the patients who had to receive prolonged (> 3 months) broad-spectrum antibiotics for other opportunistic infections, such as disseminated nontuberculous mycobacterial infections or recurrent pneumonia, because the prolonged use of antibiotics would interfere with the interpretation of the results of secondary prophylaxis against NTS bacteremia. We also excluded the patients who died during hospitalization when the first episode of NTS bacteremia developed, who were lost to follow-up within 2 months after the bacteremia, who had not received appropriate antibiotic treatment for NTS bacteremia, and who did not have sufficient laboratory data. The appropriate antibiotics for NTS bacteremia included a third- or

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fourth-generation cephalosporin, or a fluoroquinolone that demonstrated *in vitro* activity against the NTS isolate tested.

The duration of secondary prophylaxis was defined as the duration of oral antibiotics after the patients had completed the 14-day course of effective parenteral antibiotic treatment. Recurrent NTS bacteremia was defined as blood cultures that yielded NTS > 1 month after the discontinuation of antibiotic treatment or when the patient continued to receive antibiotic prophylaxis.

Rapid reduction of viremia in response to treatment was one of the important predictors of achieving long-term viral suppression.[7] Therefore, the patients included in the study were divided into two groups according to their short-term virological suppression status: the good virological suppression group, which included patients who achieved a decline of plasma HIV RNA load (PVL) by ≥ 2 $\log_{10}$ after taking 4 weeks of cART; and the poor virological suppression group, which included those who failed to achieve a decline of PVL by 2 $\log_{10}$. The patients in each group were further divided into two groups: those who received secondary prophylaxis against recurrent NTS bacteremia and those who did not.

Laboratory investigations

Isolation of *Salmonella* species was performed according to the standard methods from the blood samples. *Salmonella* serogroups B and D were further identified by the serotypes (Kauffman and White scheme), by using somatic and flagellar antigens (Denka Seiken Agglutinating Sera Salmonella Antisera Sets (Denka Seiken Co. Ltd., Tokyo, Japan)), and by conventional methods and the Phoenix System (panel type, NMIC/ID4; Becton Dickson). Disk diffusion susceptibility tests were performed for the *Salmonella* isolates, and results were interpreted according to the guidelines provided by the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards).[10] Minimum inhibitory concentrations (MICs) of ampicillin, trimethoprim/sulfamethoxazole (TMP/SMX), ceftriaxone, and ciprofloxacin were determined using the agar dilution method or VITEK 2 microbial identification system (BioMérieux, Marcy-l'Etoile, France), according to the Clinical and Laboratory Standards Institute guidelines.[10] Both of the microbiology laboratories at the National Taiwan University Hospital and Far Eastern Memorial Hospital followed the same guidelines.

CD4 lymphocyte count was determined using flow cytometry (BD FACSCalibur, Becton Dickinson and Coulter Epics XL, Beckman Coulter, Brea, CA, USA). PVL was quantified using the Cobas AmpliPrep/Cobas TaqMan HIV-I test (version 2.0; Roche Molecular Systems, Inc.) with a lower detection limit of 20 copies/mL since June 2012.

Treatment and prophylaxis

During the study period, treatment of NTS bacteremia included parenteral administration of ceftriaxone or other third-generation cephalosporins, or a fluoroquinolone, that was administered for a 14-day course, followed by oral switch to ciprofloxacin administered at a dose of 500 mg twice daily or other newer fluoroquinolones as secondary prophylaxis. The duration of fluoroquinolone prophylaxis was at the discretion of treating physicians. All patients were recommended to start cART at the time when they received a diagnosis of HIV infection with AIDS according to the national HIV treatment guidelines of the Taiwan Centers for Disease Control.[11]

Data collection

A standardized case record form was used to collect the baseline information such as age, sex, white blood cell counts, CD4 cell count, and PVL in patients with NTS bacteremia. According to the national HIV treatment guidelines, second tests for CD4 cell count and PVL will be performed 4 weeks after the initiation of cART, which are performed every 3 months subsequently during the 1st year of cART. The duration of oral TMP/SMX for the prophylaxis of *P. jirovecii* infection was also recorded.

Statistical analysis

All statistical analyses were performed using SPSS statistical software (version 20.0; SPSS Inc., Chicago, IL, USA). The variables were compared using the Mann–Whitney *U* test. The patients were followed until December 31, 2014, death, or loss to follow-up, whichever occurred first. The incidence of recurrent NTS bacteremia was calculated as the number of episodes per 100 person-years of follow-up (PYFU). Exact 95% confidence intervals (95% CI) for incidence rates were calculated on the basis of the Poisson distribution. The resistance rates of fluoroquinolone were compared using the Mid-P exact tests.

Results

Patient characteristics

During the 8-year study period, 60 patients with 68 episodes of NTS bacteremia were identified (Figure 1). In seven episodes of NTS bacteremia, the patients died during the hospitalization course; the patients was lost to follow-up soon after four episodes; in two episodes, the patients had to receive prolonged broad-spectrum antibiotics for other reasons; in one episode, NTS bacteremia was diagnosed at an outpatient clinic for which the patient did not receive appropriate antibiotic treatment; and there were no sufficient data for two episodes. After excluding these 16 episodes, 49 patients who had 52 episodes of NTS bacteremia were included for analysis.

Except for one woman, all patients were male, with a median age of 33 years and 69.4% of them being younger than 40 years (Table 1). The median CD4 cell counts at the onset of NTS bacteremia were 30 cells/μL (range, 1–140 cells/μL). For treating NTS bacteremia, one patient received a fourth-generation cephalosporin, four patients received fluoroquinolones, and others had a third-generation cephalosporin. Twenty-nine patients (59.2%) were defined as having good short-term virological response to cART, and 20 patients (40.8%) were defined as having

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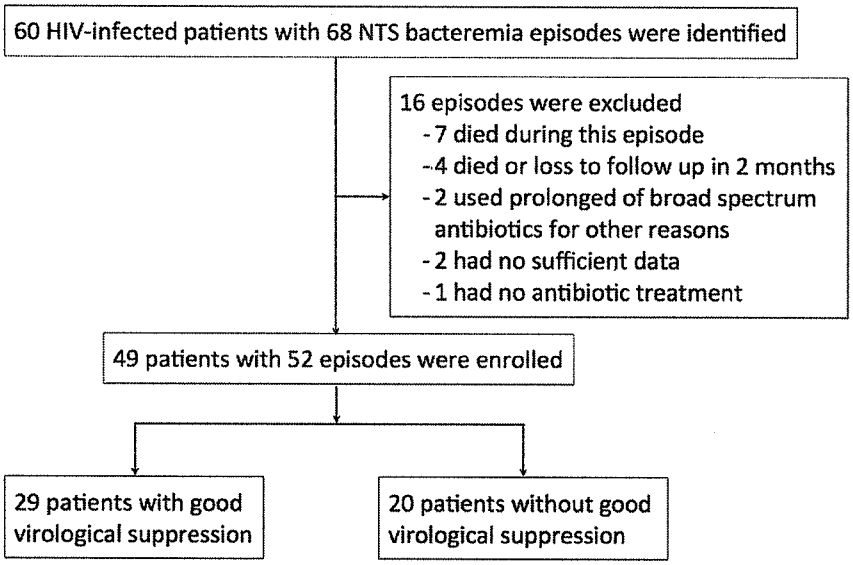


Figure 1. Disposition of patients with nontyphoid *Salmonella* (NTS) bacteremia.

poor short-term virological response. In the good virological response group, 16 patients received secondary prophylaxis and 13 did not receive prophylaxis, whereas in the poor virological response group, 15 patients received secondary prophylaxis and five did not receive prophylaxis. Twenty-four of the 49 patients (50.0%) also received TMP/SMX for the prevention of *P. jirovecii* pneumonia: 16 (16/29, 55.2%) in the good-response group (9 patients with secondary prophylaxis for NTS bacteremia and 7 without prophylaxis) and eight (8/20, 40.0%) in the poor-response group (6 patients with secondary prophylaxis for NTS bacteremia and 2 without prophylaxis). The median duration of TMP/SMX for primary or secondary prophylaxis for pneumocystosis was 28 days (range, 0–280 days) in the good-response group with prophylaxis and 0 day (range, 0–122 days) without prophylaxis; and 0 day (range, 0–154 days) in the poor-response group with prophylaxis and 0 day (range, 0–203 days) without prophylaxis (Table 1).

Immunological and virological responses

All but one patient received cART after the diagnosis of NTS bacteremia was made (Table 1). After 4 weeks of cART, the median CD4 cell count increased to 160 cells/μL (range, 12–772 cells/μL) in patients with good virological response, and 123 cells/μL (range, 3–615 cells/μL) in the poor-response group. The median follow-up duration was 1385 days (range, 330–3171 days) in the good-response group with prophylaxis and 584 days (range, 265–2968 days) without prophylaxis; and 506 days (range, 85–2826 days) in the poor-response group with prophylaxis and 526 days (range, 225–712 days) without prophylaxis (Table 2). At the end of follow-up, the median CD4 cell counts were 333 cells/μL (range, 21–731 cells/μL) in the good-response group and 61.5 cells/μL (range, 3–385 cells/μL) in the poor-response group (Table 1). In patients who had good virological response and received antibiotic prophylaxis, the CD4 cell counts at the end of follow-up were the highest among the four groups (median, 423 cells/μL; range, 80–691 cells/μL). Compared with patients with good virological response, the patients in the poor-response group, regardless of antibiotic prophylaxis for NTS bacteremia, had significantly lower median CD4 cell counts at the end of follow-up: with prophylaxis, 55 cells/μL (range, 3–385 cells/μL); without prophylaxis, 68 cells/μL (range, 3–139 cells/μL). The patients in the good-response group had a median increase of CD4 cell counts of 321 cells/μL (range, 5–678 cells/μL), whereas the CD4 count decreased by 4 cells/μL (range, −342–340 cells/μL) in the poor-response group.

The median baseline PVL was 5.38 $\log_{10}$ copies/mL (range, 4.3–6.97 $\log_{10}$ copies/mL) and 4.76 $\log_{10}$ copies/mL (range, 1.3–5.79 $\log_{10}$ copies/mL) in the good-response group and the poor-response group, respectively (Table 1). After 4 weeks of cART, the median decrease of PVL was 2.8 $\log_{10}$ copies/mL (range 2.09–3.7 $\log_{10}$ copies/mL) in patients with prophylaxis and 2.69 $\log_{10}$ copies/mL (2.15–4.04 $\log_{10}$ copies/mL) in those without prophylaxis in the good-response group, whereas it was 0.51 $\log_{10}$ copies/mL (range −3.49–1.98 $\log_{10}$ copies/mL) in patients with prophylaxis, and 0 $\log_{10}$ copies/mL (range −1.1–1.4 $\log_{10}$ copies/mL) in those without prophylaxis in the poor-response group.

Incidence and survival rate of recurrent NTS bacteremia

For the 16 patients who had good virological response and received secondary prophylaxis for NTS bacteremia, the median duration of prophylaxis was 19 days (range, 3–280 days), whereas for the 15 patients in the poor-response group who received prophylaxis, the median duration was 12 days (range, 2–52 days; Table 2). There were no recurrent episodes of NTS bacteremia in the good-response group, regardless of whether the patients received prophylaxis. In the poor virological response group, it was

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Table 1 Characteristics of 49 human immunodeficiency virus (HIV)-infected patients with nontyphoid *Salmonella* bacteremia.

Variable	Good virological response		Poor virological response		All patients
	Prophylaxis	No prophylaxis	Prophylaxis	No prophylaxis	49
	16	13	15	5	
Age (y)	31.5 (22–53)	35 (29–50)	33 (23–64)	43 (26–52)	33 (22–64)
Male	16 (100)	13 (100)	14 (93.3)	5 (100)	48 (98)
WBC count at diagnosis (cells/μL)	5145 (2140–25,090)	4750 (2540–13,360)	4500 (2130–18,690)	5240 (3570–6470)	5130 (1540–25,090)
CD4, at diagnosis (cells/μL)	15.5 (4–71)	21 (9–410)	54 (1–386)	38 (7–138)	30 (1–410)
CD4, 4 wk after taking cART (cells/μL)	153.5 (23–276)	160 (12–772)	163 (3–615)	68 (17–139)	139 (3–772)
CD4, at the end of follow-up (cells/μL)	423 (80–691)	328 (21–731)	55 (3–385)	68 (3–139)	199 (3–731)
Increase of CD4 at the end of follow-up (cells/μL)	378 (20–678)	304 (5–647)	0 (−342–340)	−12 (−35–132)	167 (−342–678)
Log_{10} PVL, at diagnosis	5.34 (4.44–6.97)	5.38 (4.3–5.89)	3.68 (1.3–5.79)	5.02 (4.71–5.33)	5.24 (1.3–6.97)
Log_{10} PVL, 4 wk after taking cART	2.55 (1.3–4.02)	2.81 (1.3–3.46)	3.67 (1.3–5.58)	5.05 (3.83–5.91)	3.12 (1.3–5.91)
Decrease of log_{10} PVL	2.8 (2.09–3.7)	2.69 (2.15–4.04)	0.51 (−3.49–1.98)	0 (−1.1–1.41)	2.31 (−3.49–4.04)
Starting HAART after bacteremia,	16 (100)	13 (100)	15 (100)	4 (80)	48 (98)
Taking TMP/SMX	9 (56.3)	7 (53.8)	6 (40)	2 (40)	24 (50)
Duration of TMP/SMX (d)	28 (0 to 280)	0 (0–122)	0 (0–154)	0 (0–203)	0 (0–280)

Data are presented as *n* (%) or median (range).
cART = combination antiretroviral therapy; HAART = highly active antiretroviral therapy; PVL = plasma HIV RNA load; TMP/SMX = trimethoprim/sulfamethoxazole; WBC = white blood cells.

5.215 per 100 PYFU (95% CI, 0.8743–17.23) with prophylaxis, and 56.42 per 100 PYFU (95% CI, 17.93–136.1) without prophylaxis.

In the poor virological response group, two patients who received prophylaxis each developed one episode of recurrent NTS bacteremia (Table 2). In the no-prophylaxis group, a patient developed one episode and another patient developed three episodes of recurrent NTS bacteremia. There was no difference between the two subgroups (receiving or not receiving prophylactic antibiotics) in the good virological response group in the incidence of recurrent NTS bacteremia, nor was there any difference between the two groups of patients who received prophylactic antibiotics and achieved good response or those who failed to achieve good virological response ($p = 0.55$).

Serotypes of NTS isolates and the antimicrobial susceptibility

Of all 52 isolates, 44 were *Salmonella* O9 (serogroup D1) and five were *Salmonella* O4 (serogroup B; Table 2). In the good virological response group, three of the 29 isolates (10.3%) were resistant to ciprofloxacin, whereas in the poor-response group, one of the 23 isolates (4.3%) was resistant to ciprofloxacin. In patients receiving a fluoroquinolone for prophylaxis, either in good or poor virological response group, two out of 32 isolates (6.3%) were resistant to ciprofloxacin. In patients without fluoroquinolone prophylaxis, two out of 20 isolates (10.0%) were resistant to ciprofloxacin ($p = 0.653$). The overall resistance rate to ciprofloxacin for all 52 isolates was 7.7% (Table 2) and the resistance rate to ampicillin, ceftriaxone, and TMP/SMX was 44.2%, 1.9%, and 26.9%, respectively.

Characteristics of patients with recurrent NTS bacteremia

Four patients accounted for six episodes of NTS bacteremia. Their clinical characteristics are shown in Table 3. All four patients had baseline CD4 cell counts < 100 cells/μL and three had CD4 cell counts < 50 cells/μL. Patient 1 had three episodes of recurrent NTS bacteremia, and died during the third episode of recurrent NTS bacteremia. His CD4 cell count was 38 cells/μL, and he had a PVL of 51,000 copies/mL when the first episode occurred. He refused to start cART despite counseling. Other than NTS bacteremia, he had disseminated *Mycobacterium avium* complex infection and *P. jirovecii* pneumonia that led to death. Patient 2 had discontinued cART for 2 years prior to admission to the hospital for cryptococcal meningitis. The CD4 cell count was 41 cells/μL, and his PVL was 111,000 copies/mL. He did not receive secondary prophylaxis for NTS bacteremia. Recurrent NTS bacteremia developed when the CD4 count decreased to 18 cells/μL. He died of cerebral septic embolism, which occurred after he received chemotherapy for subsequent anaplastic large-cell lymphoma. Patient 3 discontinued cART despite the fact that his CD4 cell count had fallen to 1 cell/μL and his PVL was 62,300 copies/mL. Ciprofloxacin was prescribed for 28 days for secondary prophylaxis. However, recurrent NTS

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Table 2 Outcome of recurrence of nontyphoid *Salmonella* bacteremia, survival, and antibiotics resistance.

Variable	Good virological response		Poor virological response	
	Prophylaxis (n = 16)	No prophylaxis (n = 13)	Prophylaxis (n = 15)	No prophylaxis (n = 5)
Duration of secondary prophylaxis (d)	19 (3–280)	0	12 (2–52)	0
Follow-up duration (d)	1385 (330–3171)	584 (265–2968)	506 (85–2826)	526 (225–712)
Having recurrent NTS bacteremia	0	0	2	2
Episodes of recurrent NTS bacteremia	0	0	2	4
Incidence, per 100 PYFU (95% CI)	0	0	5.215 (0.8743–17.23)	56.42 (17.93–136.1)
Survived	15 (39.8)	11 (84.6)	9 (60)	1 (20)
Died	0	0	2 (13.3)	3 (60)
Lost to follow-up	1 (6.3)	2 (15.4)	4 (26.7)	1 (20)
Isolates	16	13	16	7
Salmonella O9 (Group D1)	15 (93.8)	8 (61.5)	15 (93.8)	6 (85.7)
Salmonella O4 (Group B)	1 (6.3)	2 (15.4)	1 (6.3)	1 (14.3)
Antimicrobial susceptibility				
Resistant to ampicillin	6 (37.5)	8 (61.5)	8 (50)	1 (14.3)
Resistant to ciprofloxacin,	2 (12.5)	1 (7.7)	0	1 (14.3)
Resistant to ceftriaxone	0	1 (7.7)	0	0
Resistant to TMP/SMX	5 (31.3)	2 (15.4)	6 (37.5)	1 (14.3)

Data are presented as n, n (%), or median (range).
95% CI = 95% confidence interval; NTS = nontyphoid *Salmonella* bacteremia; PYFU = person-years of follow-up; TMP/SMX = trimethoprim/sulfamethoxazole.

bacteremia developed 2 months after his discharge from the hospital, and he subsequently died of *P. jirovecii* pneumonia. Patient 4 had poor adherence to cART and presented with *P. jirovecii* pneumonia, HIV nephropathy with nephrotic syndrome, and cytomegalovirus pneumonitis when NTS bacteremia occurred. NTS bacteremia recurred despite ciprofloxacin prophylaxis. He subsequently died of respiratory failure.

Discussion

In this study, we found that HIV-infected patients who had good short-term virological response to cART that was defined as a decline of PVL of $\geq$2 $\log_{10}$ after 4 weeks of cART were at a low risk of having recurrent NTS bacteremia. Regardless of the use of secondary prophylactic antibiotics, none of the 29 patients on cART with short-term virological response developed recurrent NTS bacteremia.

NTS is an important etiology of bacterial infection in immunocompromised hosts and infants in Taiwan,[12,13] and the leading cause of bacteremia in patients with advanced HIV infection.[14] The incidence of recurrent NTS bacteremia of patients without prophylaxis and poor virological suppression in the current study (56.42 per 100 PYFU) is lower than that in the pre-cART era in our previous report (70.56 per 100 PYFU).[8] The decrease may be related to the partial treatment effect of cART. For patients with good virological control, there was no recurrence of NTS bacteremia in this study (which was conducted between 2006 and 2014) compared with the incidence of 2.56 per 100 PYFU in our previous study (1997–2006).[8] Twenty-eight of the 49 patients (57.1%) were antiretroviral-naïve at the first episode of NTS bacteremia, and 42 patients (85.7%) either started cART or had changes made to the antiretroviral regimens after the episodes of NTS bacteremia. These findings highlight the importance of early diagnosis of HIV, early initiation of cART, and retention of the patients in the HIV care system for the prevention of recurrent NTS bacteremia. With good virological suppression, the role of secondary prophylaxis for NTS bacteremia may be minimized. Our findings suggest that, regardless of the baseline CD4 cell count, for patients who start cART with good adherence and virological response, prophylactic antibiotics for the prevention of recurrent NTS bacteremia may not be needed.

Patients with a low CD4 cell count are at high risk of NTS bacteremia.[15] In our previous study, we found that patients with NTS bacteremia had depleted CD4 cell counts [median, 8 cells/μL and 20 cells/μL in the pre-highly active antiretroviral therapy (HAART) and post-HAART era, respectively].[8] In this study, the patients with poor virological suppression continued to have lower CD4 cell counts at the end of follow-up (median CD4 cell count, 55 cells/μL and 68 cells/μL in patients with prophylaxis and those without prophylaxis, respectively). By contrast, patients with good virological suppression had significant increases of CD4 counts to 423 cells/μL and 328 cells/μL in patients with prophylaxis and those without prophylaxis, respectively. The CD4 cell counts at the end of follow-up of the four patients who had recurrent NTS bacteremia were all < 50 cells/μL. This suggests that a poor immunological recovery and poor adherence to cART and secondary prophylaxis prescribed may have contributed to the higher incidence of recurrent NTS bacteremia observed.[8]

In the good virological response group, nine patients received a prophylactic dose of TMP/SMX for pneumocystosis in addition to a fluoroquinolone, and seven patients received TMP/SMX without other antibiotics. None of the patients in both groups developed recurrent NTS

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Table 3 Clinical and laboratory characteristics of the four patients with recurrent nontyphoid *Salmonella* bacteremia.

Patient	1	2	3	4
Age (y)/Sex	43/M	30/M	23/M	37/M
Recurrent episodes	3	1	1	1
Salmonella serotype	O9, serogroup D1	O9, serogroup D1	O9, serogroup D1	O9, serogroup D1
Risk for HIV infection	Unknown	MSM	Unknown	MSM
Baseline CD4 count (cells/μL)	38	29.6	1	71
CD4 count at 4 wk of cART (cells/μL)	17	34.2	1	174
CD4 count at the end of follow-up (cells/μL)	3	17.5	3	40.5
Baseline PVL (copies/mL)	51,000	64,900	623,000	111,000
PVL at 4 wk of cART (copies/mL)	51,000	818,000	376,000	4630
Secondary antibiotic prophylaxis	None	None	Levofloxacin	Ciprofloxacin
Duration of prophylaxis (d)	0	0	20	12
Duration of TMP/SMX (d)	0	203	0	0
Other OIs	Disseminated MAC infection, PJP	Cryptococcal meningitis	PJP, pulmonary TB	PJP, oral candidiasis
Follow-up duration (d)	526	433	67	85

cART = combination antiretroviral therapy; MAC = *Mycobacterium avium* complex; MSM = men who have sex with men; OI = opportunistic infection; PJP = *Pneumocystis jirovecii* pneumonia; PVL = plasma HIV-RNA load; TMP/SMX = trimethoprim/sulfamethoxazole; TB = tuberculosis.

bacteremia. In the poor-response group, six patients and two patients received TMP/SMX with and without a secondary prophylactic antibiotic, respectively. Only one of four patients with recurrences (Patient 3) received TMP/SMX, with poor adherence. In our study, the resistance rate to TMP/SMX among these 52 isolates was 27.0%. With such a high resistance rate, whether receiving TMP/SMX is effective or the dose administered for preventing recurrent NTS bacteremia is appropriate remains unknown. Furthermore, the multiple adverse effects related to TMP/SMX may limit its prolonged use in the HIV-infected population.[16]

In African adults and children, the estimated mortality rate associated with NTS bacteremia was 20–25%.[17] In our study, no patient died in the good virological response group, whereas five patients (25%) died in the poor virological response group: two received antibiotic prophylaxis and three did not. However, none of these five patients died of NTS bacteremia, but of other opportunistic infections or complications. This finding suggests that the recurrence of NTS bacteremia could be a marker of poor treatment response or adherence to cART, and the patients remain at a significantly higher risk for other coexistent life-threatening opportunistic illnesses.

In our study, 44 of the 52 (84.6%) NTS isolates were *Salmonella* O9 (Group D1) and five (9.6%) were *Salmonella* O4 (Group B). In another single-center study of NTS bacteremia in both HIV-infected and non-HIV patients in northern Taiwan, serogroup D was also the most prevalent serogroup, followed by serogroup B (23.4%), serogroup C2 (6.3%), and serogroup C1 (1.6%).[12] The resistance rate to ciprofloxacin of the 52 isolates tested was 7.7%. In our previous study,[8] it was 0% in the pre-HAART era (from June 1994 to March 1997), which increased from 6.2% in the early cART era (from April 1997 to June 2002) to 34.2% in the late cART era (from July 2002 to June 2006). The causes of the observed decrease in NTS with resistance to ciprofloxacin remain to be investigated, although shortened use of fluoroquinolones in the prevention of secondary NTS bacteremia may theoretically reduce the selection pressure for the emergence of antimicrobial resistance.

Our study had several limitations. First, the number of patients is small. In view of a low incidence of recurrent NTS bacteremia in the modern cART era, comparative clinical trials of a large sample size are necessary to assess the efficacy of a short course versus longer course of prophylaxis against recurrences. Second, this is a descriptive study. Owing to the small number of patients included, the study was underpowered to demonstrate statistically significant differences in several comparisons between groups. Third, unlike our previous study,[8] we did not perform molecular typing of the *Salmonella* isolates to differentiate relapse from reinfection with NTS. Last, the patients who had poor virological response might have been infected with HIV-1 with pretreatment resistance or emergence of drug resistance mutations to the antiretroviral regimens administered; however, we did not have the data on the baseline and follow-up resistance mutations of the HIV-1 strains.

In conclusion, HIV-infected patients who achieve good short-term virological response to cART are at a low risk of having recurrent NTS bacteremia, and a prolonged course of secondary prophylactic antibiotics may not be needed.

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Conflicts of interest

All authors have no conflicts of interest to declare.

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ORIGINAL ARTICLE

Kidney dysfunction associated with tenofovir exposure in human immunodeficiency virus-1-infected Taiwanese patients

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KEYWORDS
antiretroviral therapy;
kidney dysfunction;

Abstract *Background/Purpose:* Tenofovir disoproxil fumarate (TDF) is associated with kidney tubular dysfunction, for which the risk may vary among patients of different ethnicities. Data are limited, however, on the association between renal function changes and TDF exposure in human immunodeficiency virus (HIV)-infected Taiwanese patients.

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nucleotide reverse-transcriptase inhibitor;
proximal renal tubulopathy;
tenofovir

Methods: Medical records of HIV-infected Taiwanese patients seeking HIV care at a university hospital from 2011 to 2014 were reviewed. The change of estimated glomerular filtration rate (eGFR) was compared between patients not receiving combination antiretroviral therapy (cART) and those starting cART with or without TDF. The determinants of annual eGFR changes and factors associated with greater annual eGFR decline in TDF-exposed patients were explored.

Results: A total of 775 patients were included: 140 were cART-naïve, 393 received TDF-containing cART, and 242 received cART without TDF. Compared with cART-naïve patients, the annual eGFR decline was greater in TDF-exposed patients (0.57 ± 8.6 mL/min/1.73 m^2 and 2.7 ± 8.9 mL/min/1.73 m^2, $p = 0.012$). The annual eGFR decline between patients receiving cART with or without TDF was similar (2.7 ± 8.9 mL/min/1.73 m^2 and 1.8 ± 8.3 mL/min/1.73 m^2, $p = 0.567$). Diabetes was associated with worsening eGFR decline in all studied patients. TDF exposure correlated with an additional annual eGFR decline of 2.73 mL/min/1.73 m^2 (95% confidence interval 0.139–5.326, $p = 0.039$) in patients with CD4 count < 350 cells/μL. Among TDF-exposed patients, the factors associated with annual eGFR decline of > 3 mL/min/1.73 m^2 were higher baseline eGFR and lower CD4 counts.

Conclusion: Among HIV-infected Taiwanese patients, cART exposure correlated with the decline of renal function. However, TDF-exposed patients are more likely to have prominent eGFR decline, especially those with higher baseline eGFR, advanced HIV disease, and diabetes.
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Introduction

Tenofovir disoproxil fumarate (TDF) is a widely used nucleotide reverse-transcriptase inhibitor, and is an important component of combination antiretroviral therapy (cART) for patients with human immunodeficiency virus (HIV) infection.[1,2] With the introduction of cART, survival of HIV-infected patients has significantly improved. However, aging, multiple comorbidities, complex medications, and prolonged cART may increase the risk of kidney injury. In recent years, kidney dysfunction has become a clinically relevant and important issue.[3–5]

Since its introduction for clinical use, TDF has been found to be associated with an increased risk of kidney tubular dysfunction including Fanconi syndrome, diabetes insipidus, or osteomalacia.[6,7] Decline in renal function was also reported in patients with exposure to TDF, experiencing either acute or chronic kidney injury, or merely a decrease of estimated glomerular filtration rate (eGFR) when compared with baseline values.[8]

The magnitude and clinical impact of TDF on renal function are still being debated. Variable degrees of eGFR loss have been reported, ranging from < 5 mL/min/1.73 m^2 to > 10 mL/min/1.73 m^2 annually.[8–10] In a 10-year longitudinal prospective follow-up study, there was only a mild decline of eGFR that was attributable to TDF.[11] By contrast, a study on a cohort of Japanese patients showed that the loss of eGFR increased continuously for up to 5 years.[12] Moreover, increased frequency of proteinuria has been observed in patients receiving TDF-containing cART.[13,14] Because proteinuria may often precede GFR loss, measurements of biomarkers, such as urine β-2-microglobulin, have been proposed for early detection of renal tubular dysfunction.[15]

Previous studies have shown different incidences and profiles of adverse effects of cART in Asian populations compared with those reported in Western countries.[16,17] The predictive factors of TDF-related kidney injury have been recognized, which vary among patients of different ethnicities. For Asian people, a lower weight[18,19] and certain genetic variability[20] may contribute to the development of kidney injury. A few studies have reported on the change in renal function in TDF-exposed Asians,[19,21–24] however, most of the studies had short observation periods. This study aimed to assess the eGFR changes and to identify the risk factors for decline of renal function associated with TDF exposure in HIV-1-infected Taiwanese patients.

Methods

Patient population

This retrospective cohort study was conducted between January 2011 and December 2014 at a university hospital that is the largest designated hospital for HIV care in Taiwan. Because TDF was not introduced into clinical use in Taiwan until 2011, the study population included all HIV-infected patients who regularly sought HIV care at the hospital since 2011. Three groups of patients were defined according to their treatment status: those not receiving cART, those receiving TDF-containing cART, and those receiving cART not containing TDF.

Patients were included if they were aged ≥ 20 years with at least two serum creatinine measurements with an interval of 90 days or more. The exclusion criteria included receipt of ART < 90 days, intermittent or unknown duration of ART exposure, and end-stage renal disease on dialysis. ART was initiated and prescribed according to the national treatment guidelines for HIV infection proposed by the Taiwan Centers for Disease Control.[25] The decision to

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switch or stop cART was at the discretion of the HIV-treating physicians. The study was approved by the Research Ethics Committee of National Taiwan University Hospital (registration number NTUH-201301041RIND). The data were analyzed anonymously, and written or oral informed consent was waived.

Data collection and evaluation of renal function

We used a standardized case record form to collect the information on the demographics, sexual preference, weight and height, comorbidity, treatment history of cART, plasma HIV RNA load, CD4 lymphocyte count, concomitant medications, and serum creatinine at the start of the study from the medical records of the patients. Chronic kidney disease was defined as an eGFR < 60 mL/min/1.73 m^2. Dyslipidemia was defined by regular use of lipid-lowering agents, or a total cholesterol of ≥ 240 mg/dL, or a triglycerides level of ≥ 200 mg/dL. Serum creatinine measurements were performed every 6–12 months until the study ended. All patients underwent annual proteinuria screening. If the patients discontinued TDF due to renal dysfunction, serum creatinine levels and urinalysis results were monitored and followed up until the end of study (December 31, 2014).

We used the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, incorporating serum creatinine, age, sex, and race as four parameters to estimate GFR. The CKD-EPI equation was shown to be more accurate than the Modification of Diet in Renal Disease (MDRD) equation in the subgroup with GFR > 60 mL/min/1.73 m^2.[26] Such populations include patients without kidney disease and young patients, which are very much similar to our study population. Guidelines published by the Kidney Disease Improving Global Outcomes organization, managed by the National Kidney Foundation of the United States, recommends the CKD-EPI equation for patients with higher GFR.[27] Proteinuria, detected using spot urine sample, was defined as $> 1+$ (i.e., urine protein level ≥ 30 mg/dL).

Our primary outcome of interest was the change of GFR for each group of patients. The secondary objective was to identify the risk factors associated with GFR decline in patients with TDF exposure.

Statistical analysis

Patients' demographics and basic characteristics were evaluated by descriptive statistics. Data were presented as mean (standard deviation) or count (percent). Categorical variables were compared using chi-square test or Fisher exact test. Continuous variables were compared using the Kruskal–Wallis one-way analysis of variance or Mann–Whitney *U* test. For data from two related samples, variables were compared using paired *t* test. A two-tailed *p* value < 0.05 was considered statistically significant. Factors associated with annual eGFR change in all patients were identified using multivariate linear regression model. Factors associated with annual eGFR decline by > 3 mL/min/1.73 m^2 in patients exposed to TDF were explored using the multivariate logistic regression model. Variables were entered into the model with a backward stepwise linear or logistic regression approach with p value < 0.1 as a requirement for acceptance. Data were analyzed using SPSS software version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

Clinical characteristics of patients

During the 4-year study period, a total of 775 HIV-infected patients with available serial serum creatinine data over a 90-day interval were included for analysis: 140 were not receiving cART, 393 had exposure to TDF-containing cART (TDF-exposed group), and 242 received cART not containing TDF (non-TDF-exposed group). The baseline characteristics of the patients are shown in Table 1. Overall, most patients were middle-aged homosexual men. The average weight of patients was 66.6 kg. One fourth of the patients in the TDF-exposed group had chronic hepatitis B virus infection. More patients in the non-TDF-exposed group had diabetes mellitus, hypertension, dyslipidemia, and longer duration of cART exposure with 80% of the regimens containing protease inhibitor(s). The mean follow-up duration of the patients was 672 days (standard deviation 292 days).

Renal function change of HIV patients exposed or unexposed to tenofovir

The trends of changes in eGFR in each group of patients are demonstrated chronologically by the timing of serum creatinine tests in Figure 1. In the 4-year study period, patients not starting cART had stable eGFR at around 110 mL/min/1.73 m^2. By contrast, patients receiving cART had significant decline of eGFR: a decline from 105.6 mL/min/1.73 m^2 to 97.6 mL/min/1.73 m^2 in the TDF-exposed group, and from 99.4 mL/min/1.73 m^2 to 92.7 mL/min/1.73 m^2 in the non-TDF-exposed group (Figure 1).

In Table 2, we compared the changes between the first and the last eGFR among the three groups. Both groups of patients receiving cART with or without TDF had significantly lower eGFR in the last measurements, compared with their respective first eGFR measurements (105.6 ± 16.4 mL/min/1.73 m^2 and 100.5 ± 17.1 mL/min/1.73 m^2; 99.4 ± 17.6 mL/min/1.73 m^2 and 96.4 ± 18.1 mL/min/1.73 m^2, respectively; both $p < 0.001$), however, patients not receiving cART had similar levels (110.1 ± 14.4 mL/min/1.73 m^2 and 109.8 ± 13.4 mL/min/1.73 m^2; $p = 0.387$). Compared with patients not receiving cART, the annual decline of eGFR was greater in the TDF-exposed group (0.57 ± 8.6 mL/min/1.73 m^2 and 2.7 ± 8.9 mL/min/1.73 m^2; $p = 0.012$). However, the annual declines of eGFR between the TDF-exposed group and the non-TDF-exposed group were not statistically significantly different (2.7 ± 8.9 mL/min/1.73 m^2 and 1.8 ± 8.3 mL/min/1.73 m^2; $p = 0.567$). The annual percentage of decline in eGFR was $0.1 \pm 8.1\%$ for the patients not receiving cART, which was significantly lower than that for the TDF-exposed group ($2.3 \pm 8.6\%$, $p = 0.032$) or the non-TDF-exposed group ($1.3 \pm 10.3\%$, $p = 0.035$). A urine specimen tested positive for proteinuria (protein level ≥ 30 mg/dL) in 23.2% of the patients not receiving cART, 13.9% of the patients in the TDF-exposed group, and 14.0% of the patients in the non-TDF-exposed group. The

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Table 1 Baseline characteristics of the HIV-infected patients with different treatment status.

	Not on cART (n = 140)	cART experienced, TDF exposed (n = 393)	cART experienced, non-TDF exposed (n = 242)	P
Age (y)	31.5 ± 7.3	38.2 ± 10.0	43.4 ± 12.3	<0.001
Male sex	131 (93.6)	379 (96.4)	230 (95.0)	0.345
MSM	115 (82.1)	330 (84.0)	172 (71.1)	<0.001
Injecting drug user	15 (10.7)	6 (1.5)	5 (2.1)	<0.001
Weight (kg)	67.8 ± 14.1	65.9 ± 10.7	67.0 ± 11.4	0.63
BMI (kg/m^2)	23.0 ± 4.1	22.6 ± 3.4	23.4 ± 3.4	0.038
Comorbidity				
HBsAg positive	12 (8.6)	100 (25.4)	30 (12.4)	<0.001
Anti-HCV positive	28 (20)	37 (9.4)	10 (4.1)	<0.001
Hypertension	6 (4.3)	37 (9.4)	40 (16.5)	<0.001
Diabetes mellitus	5 (3.6)	15 (3.8)	21 (8.7)	0.018
CKD [a]	0 (0)	2 (0.5)	11 (4.5)	<0.001
Malignancy	1 (0.7)	28 (7.1)	15 (6.2)	0.554
Heart failure	0 (0)	2 (0.5)	2 (0.8)	0.017
Years since HIV diagnosis	5.5 ± 2.5	7.0 ± 4.8	9.9 ± 4.9	<0.001
Duration of ART (y)	0 ± 0	5.9 ± 4.6	9.1 ± 4.9	<0.001
CD4 count (cells/μL)	541 ± 173	374 ± 291	547 ± 258	<0.001
Plasma HIV RNA load (log_{10} copies/mL)	3.96 ± 0.8	3.3 ± 1.9	1.70 ± 0.9	<0.001
Exposed to PI	0 (0)	140 (35.6)	197 (81.4)	<0.001
ACEI or ARB use	3 (2.1)	14 (3.6)	17 (7.0)	0.042
Dyslipidemia	2 (1.4)	18 (4.6)	49 (20.2)	<0.001
Follow-up duration (d)	549 ± 267	797 ± 316	541 ± 137	<0.001

[a] Defined as eGFR < 60 mL/min/1.73 m^2.
Results are presented as n (%) or mean ± standard deviation.
ACEI = angiotensin II-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; BMI = body mass index; cART = combination antiretroviral therapy; CKD = chronic kidney disease; eGFR = estimated glomerular filtration rate; HCV = hepatitis C virus; HIV = human immunodeficiency virus; MSM = men who have sex with men; PI = protease inhibitor; TDF = tenofovir disoproxil fumarate.

prevalence of proteinuria was significantly higher in the patients not receiving cART than the TDF-exposed and non-TDF-exposed groups (23.2% vs. 13.9%, p = 0.032; 23.2% vs. 14.0%, p = 0.035, respectively), but it was similar between the TDF-exposed and the non-TDF-exposed groups (13.9% vs. 14.0%, p = 0.524).

The factors influencing annual change of eGFR in HIV-infected patients were explored by multivariate linear regression (Table 3). The analysis indicated that presence of diabetes mellitus and dyslipidemia would lead to greater eGFR decrement annually at a rate of 5.01 mL/min/1.73 m^2 [95% confidence interval (CI), 1.539–7.128, p = 0.002] and 2.46 mL/min/1.73 m^2 (95% CI, 0.838–6.177, p = 0.010), respectively. On the contrary, chronic kidney disease (defined as eGFR < 60 mL/min/1.73 m^2) and every additional CD4 cell count increase would lessen the annual decrement of eGFR. TDF exposure had no significant influence on annual eGFR change.

In the subgroup analysis, we investigated the influence of TDF exposure on the annual eGFR change in patients with different CD4 levels using linear regression. In 277 patients with CD4 count < 350 cells/μL, those exposed to TDF had an additional 2.73-mL/min/1.73 m^2 eGFR decrement annually (95% CI 0.139–5.326; p = 0.039). Diabetes mellitus continued to have a significant impact on eGFR decline in this analysis (Table 3, Analysis 2).

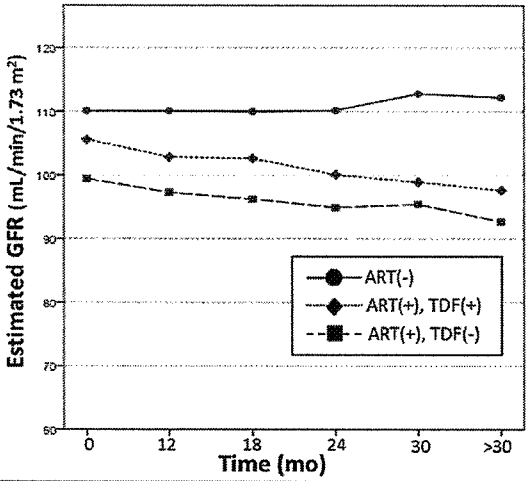
Factors associated with renal function decline in patients with TDF exposure

Analysis of the risk factors associated with annual eGFR loss >3 mL/min/1.73 m^2 in 393 TDF-exposed patients is shown in Table 4. In univariate analysis, factors associated with annual eGFR loss >3 mL/min/1.73 m^2 were increased plasma HIV RNA load and higher baseline eGFR. Patients with higher CD4 counts and longer TDF exposure appeared to have a lower rate of annual eGFR loss >3 mL/min/1.73 m^2. In multivariate logistic regression, higher baseline eGFR levels were associated with an increased risk of annual eGFR loss > 3 mL/min/1.73 m^2 [for every 10 mL/min/1.73 m^2 increase, odds ratio (OR) 1.292; 95% CI 1.123–1.486; p < 0.001], and higher CD4 counts were protective against HIV RNA (for every 1 cell/μL increase, OR 0.999; 95% CI 0.998–1.000; p = 0.008).

Outcomes of patients with TDF-related renal failure

During the study period, 11 of 393 (2.8%) patients discontinued TDF. Six patients switched to other cART regimens due to emergence of antiretroviral resistance, and five (1.3%) patients withdrew TDF due to increased serum

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Months	0	12	18	24	30	>30
ART(-), *N*	140	77	66	53	21	12
ART(+),TDF(+), *N*	393	257	284	298	289	238
ART(+),TDF(-), *N*	242	220	217	95	12	4

Figure 1. Trends of changes in estimated glomerular filtration rate (eGFR) in HIV-infected patients with three different status of combination antiretroviral therapy (cART). The three groups were as follows: patients not receiving cART [ART (−)], patients receiving TDF-containing cART [ART (+), TDF (+)], and patients receiving cART not containing TDF [ART (+), TDF (−)]. The maximal follow-up duration was 48 months. TDF = tenofovir disoproxil fumarate.

creatinine levels. The details of these five patients are shown in Table 5. Their average eGFR at baseline was 74 mL/min/1.73 m^2. At TDF discontinuation, the average loss of eGFR was 32 mL/min/1.73 m^2, and the average increase of serum creatinine levels was 0.67 mg/dL. Three patients had pre-existing hypertension or diabetes mellitus. The other two patients had no chronic illness, but their body mass indices were < 20 kg/m^2. The serum creatinine level of four patients recovered partially after TDF discontinuation (median follow-up duration 161 days). The only one patient with worsening renal function despite discontinuation of TDF was the oldest, with poorly controlled diabetes mellitus.

Discussion

In this Taiwanese cohort, the average annual decline of eGFR in TDF-exposed patients was 2.7 mL/min/1.73 m^2. In multivariate analysis, TDF exposure was correlated with an additional annual eGFR decrement of 2.73 mL/min/1.73 m^2 in patients with CD4 count < 350 cells/μL. For patients receiving TDF, the factors associated with annual eGFR decrement > 3 mL/min/1.73 m^2 were lower CD4 counts and higher baseline eGFR in multivariate analysis. The prevalence of proteinuria was higher in patients not receiving cART, but similar between patients receiving TDF- or non-TDF-containing cART. During the 4-year study period, five (1.3%) patients withdrew TDF due to deteriorating renal function.

The first study in HIV-infected Asians to evaluate change of creatinine clearance after TDF initiation was performed in Thai patients.[21] Using the Cockcroft–Gault formula and MDRD formula, the authors concluded that creatinine clearance remained stable after a median of 21 weeks of TDF exposure. Later studies in HIV-infected Japanese,[12,18,23] Chinese,[22] and Vietnamese[28] patients all suggested a harmful effect of TDF on renal function, yet expressed the result in different ways, such as TDF exposure shown to increase the risk of eGFR < 60 mL/min/1.73 m^2, eGFR > 10 mL/min/1.73 m^2 or a 25% decline of eGFR from baseline, or presence of urine markers for proximal renal tubulopathy. Overall, these studies suggested a higher risk for TDF-related renal dysfunction among Asians than the patients in Western countries.

Few studies in Asian people calculated the eGFR changes over time. Cao et al[22] reported an 8.8-mL/min/1.73 m^2 decline in eGFR at Week 48 in patients receiving both TDF and protease inhibitors.[22] Kinai and Hanabusa[23] reported a 17-mL/min/1.73 m^2 loss of eGFR at Week 96 in TDF-treated patients. The degrees of eGFR decline in these two studies are much greater compared with our observation. This could be due to the difference in observation duration. Several reports have found that eGFR of TDF-treated patients tends to decline rapidly within the first few months of TDF exposure, and then stabilizes.[22,29,30] It has been suggested that changes in eGFR may be due to inhibition of creatinine secretion of the proximal tubule due to TDF exposure rather than due to actual damages to glomerular functions.[31] When patients are followed up for longer periods, the average annual decline in eGFR would be smaller. Another factor leading to discrepancies in eGFR levels among the different studies is the equation used to estimate GFR. In HIV-infected patients with eGFR > 120 mL/min/1.73 m^2, MDRD may give higher mean eGFR estimates than CKD-EPI.[32]

Advanced HIV disease, characterized by a low CD4 count and high plasma HIV RNA load, had been recognized as a predictor of TDF-related renal function decline.[10] Current guidelines suggest initiating cART in HIV-infected individuals with a CD4 count < 500 cells/μL, and as the priority, for patients who have a CD4 count < 350 cells/μL.[1] In our study, TDF exposure was associated with a 2.73-mL/min/1.73 m^2 eGFR decline annually in patients with CD4 count < 350 cells/μL. Our results suggest that more frequent monitoring of renal function is needed in patients with advanced HIV disease preparing for initiation of TDF-based regimens. Among the factors that would increase the risk of renal dysfunction in TDF-exposed patients, a lower body weight is frequently mentioned in Asian populations.[18,28,33] The hypothesis is that a lower weight would lead to a higher TDF plasma concentration, which increases the risk of nephrotoxicity. A recent study measuring TDF plasma concentration revealed that overexposure to TDF was associated with a time-dependent decrease in eGFR.[34] In multivariate analysis, we did not find a statistically significant association between a lower weight and kidney dysfunction. However, two of the five patients with no comorbidity who discontinued TDF due to worsening renal function in our study did have a low body mass index.

Our analyses showed TDF-exposed patients with higher eGFR at baseline tend to have more prominent eGFR loss.

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Table 2 Renal function change and incidence of proteinuria of HIV-infected patients with different treatment status.

	Not on cART (n = 140)	cART experienced, TDF exposed (n = 393)	cART experienced, TDF unexposed (n = 242)	Three groups p	Not on cART vs. TDF exposed p	Not on cART vs. TDF unexposed	cART experienced, TDF vs. non-TDF
First serum Cr (mg/dL)	0.89 ± 0.14	0.89 ± 0.18	0.91 ± 0.18				
Last serum Cr (mg/dL)	0.88 ± 0.13	0.93 ± 0.19	0.94 ± 0.22				
First eGFR [a] (mL/min/1.73 m^2)	110.1 ± 14.4	105.6 ± 16.4	99.4 ± 17.6				
Last eGFR (mL/min/1.73 m^2)	109.8 ± 13.4	100.5 ± 17.1	96.4 ± 18.1				
Annual eGFR change (mL/min/1.73 m^2)	−0.57 ± 8.6	−2.7 ± 8.9	−1.8 ± 8.3	0.057	0.012	0.12	0.567
Annual eGFR change (%)	−0.1 ± 8.1	−2.3 ± 8.6	−1.3 ± 10.3	0.059	0.032	0.035	0.524
Proteinuria (≥ 30 mg/dL)	23.2% (16/69)	13.9% (47/338)	14.0% (32/235)	0.115	0.052	0.055	0.922

[a] eGFR was calculated by CKD-EPI equation.
Results are n (%), or mean ± standard deviation.
cART = combination antiretroviral therapy; CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration; Cr = creatinine; eGFR = estimated glomerular filtration rate; HIV = human immunodeficiency virus; TDF = tenofovir disoproxil fumarate.

Similar findings have also been reported previously.[18,35,36] Horberg et al[35] demonstrated that TDF-exposed patients with a baseline eGFR > 80 mL/min/1.73 m^2 had a more pronounced eGFR loss than those with baseline eGFR between 50 mL/min/1.73 m^2 and 79 mL/min/1.73 m^2. A later study from Japan found that high eGFR levels at baseline was a risk factor for a decline in eGFR by > 25%.[18] CKD patients were expected to have faster decline of renal function after initiating a nephrotoxic drug treatment. The exact reason for these conflicting data is unclear. One possible explanation is that by using the MDRD or CKD-EPI formula, patients with high eGFR had greater eGFR change than those with low eGFR in response to a same level of serum creatinine elevation. To avoid this phenomenon, methods that evaluate renal function directly, such as ^{51}Cr-EDTA clearance, might be more accurate.

Proteinuria was observed in a higher percentage of patients not receiving cART compared with the patients

Table 3 Determinants of annual change of eGFR in HIV-infected patients using multivariate linear regression.

Variable	Univariate analysis		Multivariate analysis	
	Regression coefficient (95% CI)	p	Regression coefficient (95% CI)	p
Analysis 1: All patients [a] (N = 775)				
Male sex	−3.574 (−6.493 to −0.600)	0.018		
Diabetes mellitus	−3.969 (−6.698 to −1.241)	0.004	−5.011 (−7.768 to −2.254)	<0.001
Chronic kidney disease	7.747 (2.997–12.497)	0.001	10.149 (5.403–14.895)	<0.001
Dyslipidemia	−1.986 (−4.138 to 0.165)	0.070	−2.455 (−4.610 to −0.301)	0.026
CD4 count (cells/μL)	0.004 (0.002–0.007)	<0.001	0.005 (0.003–0.007)	<0.001
Tenofovir exposure	−1.376 (−2.600 to −0.151)	0.028		
ACEI or ARB use	−2.616 (−5.608 to 0.377)	0.087		
Analysis 2: Patients with CD4 < 350 (cells/μL)[b] (N = 277)				
Diabetes mellitus	−13.862 (−20.757 to −6.967)	<0.001	−14.507 (−21.389 to −7.625)	<0.001
Tenofovir exposure	−2.246 (−4.905 to 0.412)	0.097	−2.733 (−5.326 to −0.139)	0.039

[a] In analysis 1, univariate linear regression showed no significant contribution (p > 0.1) of age, injective drug user, body weight, HBsAg-positivity, Anti-HCV-positivity, hypertension, congestive heart failure, duration of cART, plasma HIV RNA load, exposure to protease inhibitor, and follow-up duration on eGFR (not listed in the table).
[b] In analysis 2, univariate linear regression showed no significant contribution (p > 0.1) of age, injective drug user, body weight, HBsAg-positivity, Anti-HCV-positivity, chronic kidney disease, hypertension, congestive heart failure, dyslipidemia, ACEI or ARB use, duration of ART, plasma HIV RNA load, exposure to protease inhibitor, and follow-up duration on eGFR (not listed in the table).
ACEI = angiotensin II-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; cART = combination antiretroviral therapy; CI = confidence interval; eGFR = estimated glomerular filtration rate; HCV = hepatitis C virus; HIV = human immunodeficiency virus.

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Table 4 Determinants of annual decline of eGFR by ≥ 3 mL/min/1.73 m^2 in HIV-infected patients treated with tenofovir.

	Annual decline of eGFR		Univariate analysis		Multivariate analysis	
	≥ 3 mL/min/1.73 m^2 (n = 146)	< 3 mL/min/1.73 m^2 (n = 247)	OR (95% CI)	p	OR (95% CI)	p
Age (Y) [a]	38.8 ± 11.1	37.8 ± 9.3	1.009 (0.989–1.030)	0.363		
Male sex	143 (97.9)	236 (95.5)	2.222 (0.61–8.099)	0.226		
Weight < 50 kg	11 (7.5)	13 (5.5)	1.423 (0.62–3.268)	0.406		
HBsAg-positive	32 (21.9)	68 (27.5)	0.739 (0.457–1.196)	0.218		
Anti-HCV-positive	11 (7.5)	26 (10.5)	0.693 (0.332–1.447)	0.328		
Hypertension	17 (11.6)	20 (8.1)	1.496 (0.756–2.957)	0.247		
Diabetes mellitus	8 (5.5)	7 (2.8)	1.988 (0.706–5.600)	0.194		
Malignancy	11 (7.5)	17 (6.9)	1.102 (0.502–2.423)	0.808		
Congestive heart failure	1 (0.7)	1 (0.4)	1.697 (0.105–27.33)	0.709		
Dyslipidemia	5 (3.4)	13(5.3)	0.638 (0.223–1.828)	0.403		
CD4 count (cells/μL) [a]	308 ± 294	412 ± 282	0.999 (0.998–0.999)	0.004	0.999 (0.998–1.000)	0.008
HIV PVL (log_{10} copies/mL) [a]	3.6 ± 1.9	3.1 ± 1.8	1.156 (1.035–1.291)	0.010		
Exposure to PI	54 (37.0)	86 (34.8)	1.099 (0.718–1.682)	0.665		
ACEI or ARB use	6 (4.1)	8 (3.2)	1.280 (0.435–3.766)	0.653		
Tenofovir exposure (d) [a]	736 ± 178	833 ± 332	0.999 (0.998–1.000)	0.003	1.000 (0.999–1.000)	0.186
Baseline eGFR (mL/min/1.73 m^2) [a]	110.2 ± 16.4	102.9 ± 15.9	1.342 (1.168–1.542)	<0.001	1.292 (1.123–1.486)	<0.001

[a] For continuous variable in logistic regression, the odds ratios are shown for each 1-year increase in age, for each 1-cell/μL increase of CD4 count, for each 1 log_{10} copy/mL increase of PVL, for each 1-day increase of TDF exposure, and for each 10-mL/min/1.73 m^2 increase of baseline eGFR.
ACEI = angiotensin II-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; CI = confidence interval; eGFR = estimated glomerular filtration rate; HCV = hepatitis C virus; HIV = human immunodeficiency virus; OR = odds ratio; PI = protease inhibitor; PVL = plasma HIV-RNA load; TDF = tenofovir disoproxil fumarate.

receiving cART in our study. This is not unexpected because with the introduction of cART, there has been a decreasing incidence of HIV-associated nephropathy. A previous study also showed that cART initiation was associated with improvement in proteinuria.[37] Being a simple laboratory test, urine dipstick test is recommended for screening proteinuria in TDF-treated patients.[38] One study that included 10,841 HIV-infected patients reported that 1 additional year of TDF exposure was associated with 34% increased risk of proteinuria.[39] Limited by the small sample size of our study, we did not find a statistically significant difference in the prevalence of proteinuria between the TDF-exposed and non-TDF-exposed groups.

Five (1.3%) patients withdrew TDF due to increased serum creatinine levels during the study period. The average increase in serum creatinine levels was 0.67 mg/dL at TDF discontinuation. In a cohort study of 10,343 HIV-infected patients receiving TDF-containing cART, 2.2% of patients had an increase in serum creatinine levels of ≥ 0.5 mg/dL, and 0.5% experienced a serious renal adverse event of any type.[40] A more recent study in Thailand reported that 41 of 1204 (3.4%) TDF-treated patients had an increase in serum creatinine level of ≥ 0.5 mg/dL from baseline.[41] Published guidelines suggest obtaining measurements of serum creatinine levels consistently for TDF-treated patients.[38] However, there is no consensus on the optimal timing to discontinue TDF in patients whose kidney function declines progressively, and the best marker for TDF-related kidney injury has yet to be defined. After discontinuation of TDF, four of the five patients in this study had their eGFR partially recovered, which is in line with the previous studies showing that the loss of renal function may not be fully reversible with TDF withdrawal.[42]

There are several limitations of our study and our results should be interpreted with caution. First, this is a retrospective study. Patients included in our study might not have a uniform schedule of blood sampling, and their adherence to cART might be incomplete. Second, although we provided a relatively longer observation period than previous studies in Asia,[21,22] the duration of TDF exposure was no more than 4 years. Because the pattern of eGFR decline may not be linear, the changes of renal function in the short-term observation period may not predict the long-term clinical effect. Third, we did not examine other parameters representing renal tubular dysfunction, such as glycosuria, urine phosphate, or urinary β2-microglobulin. Likewise, we assessed proteinuria only qualitatively. Measurement of microalbuminuria or urine protein-to-creatinine ratio would more precisely reflect the urine protein loss. Finally, HIV-infected women and patients with a low eGFR comprised only a small proportion of our study populations. Whether our findings can be generalized to these patients warrants further investigations.

In conclusion, cART exposure correlated with the decline of renal function among HIV-infected Taiwanese patients. However, TDF-exposed patients are more likely to have

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Table 5 Details of the patients who discontinued tenofovir due to worsening renal dysfunction.

Patient No.	Age/Sex	Weight (kg)/BMI (kg/m^2)	Comorbidity	Concomitant ART	CD4 (cells/μL)	TDF duration (d)	Baseline Cr (mg/dL)	Baseline eGFR (mL/min/1.73 m^2)	Maximal Cr	Maximal eGFR	Protein in urinalysis	After TDF withdrawal Cr (mg/dL)	After TDF withdrawal eGFR
1	40/M	55/18.2	Nil	3TC/LPVr	242	663	0.9	106	1.6	53	30 (1+)	1.3	67
2	43/M	67/22.3	HTN	3TC/EFV	497	869	1.5	53	1.9	42	Negative	1.5	55
3	50/M	73/26.1	HTN, DM	3TC/NVP	15	530	1.2	70	1.8	42	100 (2+)	1.7	45
4	54/M	60/19.2	Nil	3TC/RAL	13	311	1.1	76	1.9	39	30 (1+)	1.8	41
5	75/M	62/24.2	HTN, DM	3TC/LPVr	410	738	1.1	65	1.9	33	50 (1+)	2.2	28

3TC = lamivudine; ART = antiretroviral therapy; BMI = body mass index; Cr = creatinine; DM = diabetes mellitus; EFV = efavirenz; eGFR = estimated glomerular filtration rate; HTN = hypertension; LPVr = lopinavir/ritonavir; M = male; NVP = nevirapine; RAL = raltegravir; TDF = tenofovir disoproxil fumarate.

prominent eGFR decline, especially those with advanced HIV disease (lower CD4 and high HIV RNA), diabetes mellitus, and higher baseline eGFR levels. Our results highlight the importance of renal function monitoring when starting TDF in patients initiating cART. As the survival rates of HIV-infected patients are approaching that of the general population in the cART era, the impact of prolonged TDF exposure on renal function should be carefully monitored.

Conflicts of interest

C.-C. H. has received research support from Janssen and speaker honoraria from AbbVie, Bristol-Myers Squibb, Gilead Sciences, and ViiV, and served on advisory boards for Gilead Sciences and AbbVie. All other authors have no conflicts of interest to declare.

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