HIV and hepatitis virus co-infection among injecting drug users in West Java, Indonesia

Azzania Fibriani

The research described in this thesis was conducted at the Viroscience lab of the Erasmus MC, the Netherlands and at the Hasan Sadikin hospital.Financial support for this research was obtained from a Japan International Presidential Scholarship (JIPS) from the World Bank.

Financial support for printing of this thesis from Viroclinics Biosciences B.V.

ISBN: 978-90-8891-965-7
Cover design: Avia Hadi
Printed & Lay Out by: Proefschriftmaken.nl || Uitgeverij BOXPress
Published by: Uitgeverij BOXPress, 's-Hertogenbosch

HIV and hepatitis virus co-infection among injecting drug users in West Java, Indonesia

HIV en hepatitis co-infecties bij intraveneuze drug gebruikers in West Java, Indonesië

Proefschrift

ter verkrijging van de graad van doctor aan de

Erasmus Universiteit Rotterdam

op gezag van de

rector magnificus

Prof. Dr. H. A. P Pols

en volgens besluit van het College voor Promoties

De openbare verdediging zal plaatsvinden op

17 Oktober 2014 om 11.30 uur

Door

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geboren te Bandung, Indonesië

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Contents

Chapter 1

Update of HIV and hepatitis virus co-infection in Indonesia (General Introduction)

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Submitted

Abstrak

Saat ini, Indonesia merupakan negara dengan pertumbuhan epidemiologi HIV tercepat se-Asia. Di lain pihak, koinfeksi hepatitis B dan C merupakan hal yang umum bagi pasien yang terinfeksi HIV. Namun demikian, sampai saat ini belum ada penelitian yang mendalam mengenai koinfeksi HIV dan hepatitis. Oleh karena itu, dalam kajian ini kami akan mecoba memaparkan informasi terkini mengenai koinfeksi HIV dan hepatitis di Indonesia. Ringkasan ini akan meliputi prevalen dan distribusi genotip dari virus hepatitis pada penderita HIV. Selanjutnya, akan dipaparkan juga mengenai tantangan yang ada dalam diagnosis, pengobatan dan pengontrolan HIV dan virus hepatitis pada pasien di Indonesia. Pengadaan obat hepatitis dengan harga terjangkau dan tes diagnostik yang murah merupakan syarat penting untuk meningkatkan kualitas pengobatan pada pasien yang terkoinfeksi HIV dan hepatitis.

Kata kunci: Indonesia, HIV, hepatitis

Abstract

Indonesia currently has the fastest growing HIV epidemic among Asian countries. Moreover, co-infections with hepatitis B and C virus are not uncommon among HIV infected-patients. Comprehensive information about these co-infections is scarce. In this article, we summarize information of HIV and hepatitis co-infections in Indonesia. The co-infection prevalence as well as the viral genotypic distribution of viral hepatitis is described among HIV-infected individuals. Challenges that exist in diagnosing, treating and monitoring of HIV and viral hepatitis co-infected patients in developing countries are discussed. Further work in providing affordable drugs and cheaper monitoring tools are needed in order to increase the number of co-infected patients receiving treatment.

Keywords: Indonesia, HIV, hepatitis

Introduction

Worldwide, HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV) are ranked among the top 10 major causes of infectious disease death.[1] Due to shared routes of transmission, co-infection of HIV with hepatitis virus is relatively common. Of people living with HIV, 20% (7 million) of them have chronic hepatitis C infection and 9% (3 million) have chronic hepatitis B infection.[2]

Indonesia currently has the fastest growing HIV epidemic among Asia.[3] Compared to other countries such as Malaysia, Myanmar and Thailand that have recently showed decreasing trends in HIV incidence, Indonesia has had a three-fold increase in prevalence within one year (from <0.1% in year 2011 into 0.3% in 2012).[4]

Indonesia has a high burden of HBV infection ranging from 5 to 20% in the provinces.[5] The HCV prevalence is relatively low in the general population (2-3%)[6], but the prevalence has been reported to reach as high as 87% in patients with a history of intravenous drug use (IDU).[7] In this review, we summarize the situation of HIV and hepatitis co-infection in Indonesia.

Diagnosis

Diagnosis of HIV

In Indonesia, an algorithm employing three different serologic tests is used to diagnose HIV infection.[8] As the first test, an immunoassay with a high sensitivity (>99%) is used, while in the second and the third tests, immunoassays with high specificities are used (≥99%). HIV infection is diagnosed if all three assays give a positive result; in this case, a patient should be referred to the care support and treatment program. In contrast, if the first and second tests are positive, but the third test is negative; or, if only the first test is positive, while the second and the third tests are negative, the patient will be categorized as having an indeterminate HIV infection and the HIV test should be repeated within one month. A negative result or no HIV infection is defined as an undetectable HIV antibody result in all three assays used.

A previous study by Indrati *et al* (2009) demonstrated the possibility to use a dual testing algorithm for HIV infection diagnosis, especially for the high-risk population.[9] This study was conducted in Hasan Sadikin hospital, a referral hospital for West Java. Between 2006 and

2008 as many as 3121 patients were tested with three HIV immunoassays. A false positive result was considered if there was a discrepancy between the first and either the second or the third test. This study demonstrated that the initial rapid HIV-test did not show any false positive results and no significant differences were found between the second and third tests. Therefore it was suggested that instead of using the three test algorithm, dual testing algorithm be used instead, as this algorithm also gives adequate results for accurate detection of HIV infection in patients, especially in those patients from high risk group.

To date, in addition to immonunoassays, viral load tests which measure the amount of viral RNA in body fluids can also be used for HIV diagnosis. Due to the high sensitivity of viral load tests, they are used for HIV treatment monitoring to measure therapy efficacy, and also to diagnose HIV infection in infants born to HIV positive mothers.[10] Nevertheless, the high costs of viral load assays limits their implementation into routine care in most developing countries such as Indonesia. Subsequently, there is a need to develop an accurate and cost-effective viral load assay to increase the effectiveness of HIV therapy.

Diagnosis of hepatitis virus

Generally, diagnosis of hepatitic disease is made by biochemical assessment of liver function such as bilirubin, alaninetransferase (ALT) or aspartatetransferase (AST), alkaline phosphatase, prothrombin time, total protein, albumin, serum globulin, complete blood count, and coagulation.[11] Subsequently, HBV diagnosis is confirmed by specific antigen or antibody tests. There are three antigen-antibody systems that are useful for identifying HBV infection. The first is hepatitis B surface antigen (HBsAg) and HBsAg antibody (anti-HBS). The second is hepatitis B core antigen antibody (anti-HBc). The third is hepatitis e antigen (HbeAg) and HbeAg antibody (anti-Hbe).[12] HBsAg detection appears in early active acute infection and remains in the serum from several weeks before onset of symptoms to months after onset. As HBV acute infection is resolving, anti-HBS replaces HBsAg. Persistance of HBsAg detection in serum indicates a chronic HBV infection.[13] Anti-HBc is the first type of antibody that emerges after HBV exposure. The existence of anti-HBc in serum indicates current or past HBV infection.[11] Anti-HBe appears after anti-HBc and the presence of anti-HBe is associated with a

decline of viral infectivity. In the resolution of hepatitis disease, anti-HBe replaces HBeAg.[11,12] However, diagnosis of HBV infection in HIV-infected patients can be complicated because HIV infection may change the natural course of HBV infection.[14] First, spontaneous reverse seroconversion which is reflected by the disappearance of anti-HBS and the reappearance of HBsAg can occur especially in patients with lower CD4 cells counts (below 200 cells/mm^3). Therefore, in HIV-infected individuals with a prior detection of anti-HBS, the HBV serological testing should be repeated if unexpected liver disease occurs.[14] Second, occult HBV infection (OBI) which is defined as the presence of HBV-DNA, but the absence of HBsAg in serum patients, is often found in HIV-infected patients. OBI prevalence in HIV-patients ranges from 4.5% to 11.3%[15-17], slightly higher that the OBI prevalence in healthy blood donors.[18] Some studies have reported a strong association between the OBI and anti-HBc positivity in HIV-infected patients.[15, 16] Thus, it might be useful to perform a HBV-DNA test in HIV patients who have anti-HBc detection.

In case of HCV, diagnosis can be performed using serogical test or molecular assays. In the serological test, antibody is used to detect hepatitis C virus in serum or plasma (anti-HCV), while in the molecular assay, HCV-RNA in infected patients are quantified using nucleic acid detection tests such as a PCR-based or real-time PCR-based assays.[19-21] In HIV-infected patients, the anti-HCV antibody response might be delayed until more than 9 months after the first infection[22], molecular assays may therefore be useful in HIV patients in order to diagnose acute HCV infection.

Prevalence

The HIV and viral hepatitis co-infection prevalence is summarized in Table 1. The first study on HIV and hepatitis co-infection was reported by Wisaksana *et al.* (2010). This study was conducted at the Hasan Sadikin hospital, a referral hospital for HIV in West Java. A cross-sectional study has been conducted among HIV-positive patients who were more than 14 years old and presented between 1996 and 2008. Of 773 HIV-positive patients, the prevalence of HBsAg was 7%, while the prevalence of HCV-antibody was 71%. HCV was mainly found in patients with a history of IDU. The HCV co-infection prevalence reached as high as 87%.[7]

Comparable results were obtained from other studies that were conducted in the main narcotic prison of West Java[23] and in Dr. Sardjito hospital, a referral hospital for East Java.[24] Remarkably, higher HBV/HIV co-infection prevalence was found in a study from a private clinic in East Java.[25] In this study, the HBV/HIV co-infection prevalence was twice as high as compared with previous studies (15%).[7, 23, 24]

The route of HBV transmission among HIV-infected patients remains unclear, however previous studies have shown that vertical or early childhood transmission play major roles in most of developing countries.[26] On the other hand, unlike HCV transmission in the general population[6], HCV transmission in HIV-infected patients is likely dominated by IDU.[24]

Genotypic distribution

Genotype distribution in HBV/HIV co-infected patients

In 2010, Anggorowati *et al.* performed HBV genotyping based on the *S* gene region in two HIV patients who were also positive for HBsAg at Dr. Sardjito Hospital, Central Java.[24] Phylogenetic analysis showed that both patients had HBV sub genotype B3, which is the most common HBV sub genotype in Indonesia, particularly on Java Island.[5] The same genotype was detected by Utsumi *et al.* (2013) who found HBV sub genotype B3 followed by sub genotype C1 as the major prevalent genotypes in HBV/HIV co-infected patients.[25]

HCV genotypic distribution in HCV/HIV co-infected patients

In case of HCV, genotyping was performed using *NS5B,* a RNA-dependent polymerase gene. Based on *NS5B* fragment analysis, at least seven genotypes were found in 77 HIV positive patients.[24] The major prevalence was sub genotype 1a (23/77), followed by 3k (7/77), 1c (5/77), 4a (4/77), 1b 1/77) and 6n (1/77). Previous studies[6, 27, 28] however, showed that unlike HIV-infected patients, sub genotype 1b was the most prevalent genotype in the general population. The differences in HCV sub genotypes between HIV infected patients and general patients might influence the HCV treatment outcome response between those two populations.

Treatment and monitoring

HIV treatment in hepatitis virus co-infected patients

The 2011 national HIV treatment guidelines for adults (Tatalaksana klinis infeksi HIV dan terapi antiretroviral pada orang dewasa) recommends initiating antiretroviral therapy (ART) in patients with active HBV infection regardless of their CD4 cell count. For this purpose, antiretrovirals that have dual activities against HBV and HIV, such as tenofovir (TDF) plus lamivudine (3TC) or emtricitabine (FTC), should be used.[8] Subsequently, in 2013, the Minister of Health improved the treatment recommendation for HIV and HBV and/or HCV co-infection. In contrast with the previous guidelines (2011), the new decree recommends ART initiation in all HIV and HBV co-infected patients, regardless of their HBV status.[29] Because not all HIV and HBV co-infected individuals carry active HBV infection, the efficacy of ART in non-active HBV infected patient merits further evaluation.

Generally, HIV therapy for people with and without HCV infection is the same, however, some HIV regimens such as zidovudine and stavudine are not recommended for co-infected patients under HCV therapy. The used of zidovudine or stavudine together with an HCV drug such as ribavirin may induce hepatotoxicity in treated individuals. Therefore, it is recommended to substitute these HIV drugs with tenofovir during HCV therapy.[8]

Despite a high mortality rate which is usually found in HIV and viral hepatitis co-infection, virological response to highly active antiretroviral therapy (HAART) was comparable between HIV patients with and without viral hepatitis infection.[30] However, significantly lower CD4+ cell recovery was more often found in HIV/HBV[31, 32] and HIV/HCV[33] co-infected patients than in HIV mono-infected patients. On the other hand, the impact of viral hepatitis co-infection on HAART treatment outcome in Indonesian patients has not been reported using a large cohort study. Further work therefore needs to be performed in the future to address this particular issue.

HBV treatment in /HIV co-infected patients

As mentioned above, the 2011 national HIV treatment guidelines for adults (Tatalaksana klinis infeksi HIV dan terapi antiretroviral pada orang dewasa) recommends the use of tenofovir combined with either lamivudine or emtricitabine for treating HBV and HIV co-infected patients.

Unfortunately, treatment of HBV/HIV co-infected patients from Indonesia has not been properly evaluated, yet most studies advised to monitor HBV-DNA and ALT level every three months.[14] Frequent monitoring allows for early detection of the appearance of drug resistance mutations. However, in most HBV/HIV co-infected patients in this country, the high cost of the HBV-DNA test might become a barrier for proper treatment monitoring. Therefore an affordable HBV-DNA test for these patients in the future is urgently needed.

HCV treatment in HIV co-infected patients

Based on the 2011 national HIV treatment guideline for adults[8], HCV treatment initiation was recommended in patients who have a CD4 count >350 cells/mm^3 and have HIV viral suppression after ART. Pegylated interferon (pegIFN) alpha 2A or 2B in combination with ribavirin is prescribed for HCV treatment in HIV co-infected patients. In pegIFN alpha 2A plus ribavirin, the pegIFN alpha 2A dose is 180 µg/week and ribavirin dose is 1000 mg for body weight <75 kg and 1200 mg for body weight >75 kg. In pegIFN alpha 2B plus ribavirin, the pegIFN alpha 2B dose is 1.5 µg/week and ribavirin dose is 800 mg for body weight <65 kg and 1200 mg for body weight >65 kg. The treatment duration is different by HCV genotype, 24 weeks in HCV genotype 2 and 3, and 48 weeks in HCV genotype 1 and 4.

Baseline CD4 cell counts may be a critical factor in HCV treatment initiation. A study in West and East Java demonstrated that most HCV/HIV co-infected patients had CD4 cell counts below 200 cells/mm.[37, 24] On the other hand, it is recommended that patients who receive HCV therapy should have a CD4 cell counts greater than 200 cells/mm^3, mainly due to the toxicities of pegIFN and/or ribavirin as well as an increased frequency of insufficient responses among severely immune deficient patients.[34] Therefore, in HIV untreated patients who had CD4 <200 cells/mm^3, it is recommended to start ART immediately. Once the CD4 cell count has increased and plasma HIV-RNA is undetectable, HCV therapy can be initiated.[35]

In patients who initiate HCV therapy, the national guideline recommends monitoring liver enzymes once a week within one month and to measure HCV-RNA at the 4th, 12th, 24th and 48th week after therapy.[8] End-of-treatment response (ETR) is defined as undetectable HCV-RNA after 24 or 48 weeks therapy, while sustained virological response (SVR) is defined

as undetectable HCV-RNA at 24 weeks after treatment completion. Unfortunately, the HCV treatment outcome in HCV/HIV co-infected patients in Indonesia has not been properly studied. However, some studies have reported the efficacy of HCV therapy in HCV mono-infected patients in Indonesia that could be extrapolated to treatment efficacy in HCV/HIV co-infected patients. Simandjuntak *et al.* (2002) reported high-dose of interferon alpha 2B therapy outcomes from 15 HCV mono-infected patients in Pertamina Central hospital, Jakarta.[36] All patients received 6 MIU of interferon alpha 2B for 24 weeks. Adequate response was observed; at the end of therapy 66% of patients had undetectable HCV-RNA. However, more side effects such as fever, fatigue, myalgia, headache and anorexia were found with high-dose prescription of interferon 2B in HCV mono-infected patients. In another study, Akbar *et al*. (2009) evaluated the response of interferon alpha 2B in combination with ribavirin in 30 HCV mono-infected patients from four hospitals in Jakarta in 2004.[37] Among those 30 patients, 23 were HCV genotype 1 and 4 (14 genotype 1 and 9 genotype 4), while 7 had HCV genotype others than 1 and 4 (3 genotype 2, 2 genotype 3 and 1 genotype 10). All participants received 3 MIU of interferon alpha 2B subcutaneously three times per week and 800-1200 mg daily ribavirin; treatment duration was 48 weeks. Good response was obtained in all HCV genotypes. However, compared to HCV genotype 1 and 4, higher rate of SVR was observed in HCV genotypes 2, 3 and 10 (69% *vs.* 100%). This study was comparable with other studies in HCV/HIV co-infected patients where successful pegIFN and ribavirin treatment rate was higher in HCV genotypes 2 and 3 than in genotypes 1 and 4.[38] However, like in HBV/HIV co-infected patients, most of HCV/HIV co-infected patients could not afford HCV treatment and monitoring (HCV-RNA test). Therefore, there is an urgent need to provide low cost HCV drugs and cheaper HCV-RNA tests to eradicate HCV infection, especially among HIV-infected patients.

Conclusion

Comparable HBV prevalence was observed in both HIV-infected patients and in the general population. Higher prevalence of HCV, however, was observed in HIV-infected patients than in the general population. Lack of clinical evidences in Indonesia makes it difficult to evaluate the treatment efficacy among HIV and viral hepatitis co-infected individuals. Therefore, affordable

drugs and monitoring tools are needed to increase the number of patients on treatment, particularly among viral hepatitis co-infected patients.

References

1. Alter MJ. Epidemiology of viral hepatitis and HIV co-infection. J Hepatol 2006;44(1 Suppl):S6-9.
2. Soriano V, Puoti M, Peters M, Benhamou Y, Sulkowski M, Zoulim F, et al. Care of HIV patients with chronic hepatitis B: updated recommendations from the HIV-Hepatitis B Virus International Panel. AIDS 2008;22(12):1399-410.
3. UNAIDS. Global report: Unaids report on the global aids epidemic 2010. Switzerland: unaids.org; 2010.
4. UNAIDS. Global Report: UNAIDS report on the global AIDS epidemic 2013. Switzerland: unaids.org; 2013.
5. Mulyanto, Depamede SN, Surayah K, Tsuda F, Ichiyama K, Takahashi M, et al. A nationwide molecular epidemiological study on hepatitis B virus in Indonesia: identification of two novel subgenotypes, B8 and C7. Arch Virol 2009;154(7):1047-59.
6. Utama A, Budiarto BR, Monasari D, Octavia TI, Chandra IS, Gani RA, et al. Hepatitis C virus genotype in blood donors and associated liver disease in Indonesia. Intervirology 2008;51(6):410-6.
7. Wisaksana R, Indrati AK, Fibriani A, Rogayah E, Sudjana P, Djajakusumah TS, et al. Response to first-line antiretroviral treatment among human immunodeficiency virus-infected patients with and without a history of injecting drug use in Indonesia. Addiction 2010;105(6):1055-61.
8. Kementrian kesehatan Republik Indonesia Direktorat Jendral pengendalian penyakit dan penyehatan lingkungan. Pedoman nasional tatalaksana klinis infeksi HIV dan terapi antiretroviral pada orang dewasa. Jakarta, Indonesia: Kementrian kesehatan Republik Indonesia Direktorat Jendral pengendalian penyakit dan penyehatan lingkungan 2011.
9. Indrati AR, van Crevel R, Parwati I, Tjandrawati A, Noormartany, Kurmalawati J. Screening and diagnosis of HIV-infection in Indonesia: one, two or three tests? Acta Med Indones 2009;41 Suppl 1:28-32.
10. Preiser W, Drexler JF, Drosten C. HIV-1 viral load assays for resource-limited settings: clades matter. PLoS Med 2006;3(12):e538; author reply e550.
11. WS R, editor. Hepatitis B virus and hepatitis D virus. New York: Churchill Livingstone; 1995.
12. Hollinger FB LT. Hepatitis B Virus. Philadelphia: Lippincott Williams & Wilkins; 2001.
13. Mahoney FJ KM, editor. Hepatitis B vaccine. Philadelphia: W.B. Saunders Company.

14. Thio CL. Hepatitis B and human immunodeficiency virus coinfection. Hepatology 2009;49(5 Suppl):S138-45.
15. Tramuto F, Maida CM, Colomba GM, Di Carlo P, Vitale F. Prevalence of occult hepatitis B virus infection in a cohort of HIV-positive patients resident in Sicily, Italy. Biomed Res Int 2013;2013:859583.
16. Chadwick D, Doyle T, Ellis S, Price D, Abbas I, Valappil M, et al. Occult hepatitis B virus coinfection in HIV-positive African migrants to the UK: a point prevalence study. HIV Med 2013.
17. Hamers RL, Zaaijer HL, Wallis CL, Siwale M, Ive P, Botes ME, et al. HIV-HBV coinfection in Southern Africa and the effect of lamivudine- versus tenofovir-containing cART on HBV outcomes. J Acquir Immune Defic Syndr 2013;64(2):174-82.
18. Vaezjalali M, Rashidpour S, Rezaee H, Hajibeigi B, Zeidi M, Gachkar L, et al. Hepatitis B viral DNA among HBs antigen negative healthy blood donors. Hepat Mon 2013;13(3):e6590.
19. Gretch DR. Diagnostic tests for hepatitis C. Hepatology 1997;26(3 Suppl 1):43S-47S.
20. Meng S, Li J. A novel duplex real-time reverse transcriptase-polymerase chain reaction assay for the detection of hepatitis C viral RNA with armored RNA as internal control. Virol J 2010;7:117.
21. Sarrazin C, Gartner BC, Sizmann D, Babiel R, Mihm U, Hofmann WP, et al. Comparison of conventional PCR with real-time PCR and branched DNA-based assays for hepatitis C virus RNA quantification and clinical significance for genotypes 1 to 5. J Clin Microbiol 2006;44(3):729-37.
22. Thomson EC, Nastouli E, Main J, Karayiannis P, Eliahoo J, Muir D, et al. Delayed anti-HCV antibody response in HIV-positive men acutely infected with HCV. AIDS 2009;23(1):89-93.
23. Nelwan EJ, Van Crevel R, Alisjahbana B, Indrati AK, Dwiyana RF, Nuralam N, et al. Human immunodeficiency virus, hepatitis B and hepatitis C in an Indonesian prison: prevalence, risk factors and implications of HIV screening. Trop Med Int Health 2010;15(12):1491-8.
24. Anggorowati N, Yano Y, Heriyanto DS, Rinonce HT, Utsumi T, Mulya DP, et al. Clinical and virological characteristics of hepatitis B or C virus co-infection with HIV in Indonesian patients. J Med Virol 2012;84(6):857-65.
25. Utsumi T, Yano Y, Lusida MI, Nasronudin, Amin M, Juniastuti, et al. Detection of highly prevalent hepatitis B virus co-infection with HIV in Indonesia. Hepatol Res 2013;43(10):1032-9.
26. Thio CL, Locarnini S. Treatment of HIV/HBV coinfection: clinical and virologic issues. AIDS Rev 2007;9(1):40-53.

27. Utama A, Tania NP, Dhenni R, Gani RA, Hasan I, Sanityoso A, et al. Genotype diversity of hepatitis C virus (HCV) in HCV-associated liver disease patients in Indonesia. Liver Int 2010;30(8):1152-60.
28. Utama A, Octavia TI, Dhenni R, Miskad UA, Yusuf I, Tai S. Hepatitis B virus genotypes/subgenotypes in voluntary blood donors in Makassar, South Sulawesi, Indonesia. Virol J 2009;6:128.
29. Indonesia MKR. Surat edaran no 129 tahun 2013 tentang pelaksanaan pengendalian HIV-AIDS daninfeksi menular (IMS). In; 2013.
30. Zhou J, Dore GJ, Zhang F, Lim PL, Chen YM. Hepatitis B and C virus coinfection in The TREAT Asia HIV Observational Database. J Gastroenterol Hepatol 2007;22(9):1510-8.
31. Wandeler G, Gsponer T, Bihl F, Bernasconi E, Cavassini M, Kovari H, et al. Hepatitis B virus infection is associated with impaired immunological recovery during antiretroviral therapy in the Swiss HIV cohort study. J Infect Dis 2013;208(9):1454-8.
32. Ladep NG, Agaba PA, Agbaji O, Muazu A, Ugoagwu P, Imade G, et al. Rates and impact of hepatitis on human immunodeficiency virus infection in a large African cohort. World J Gastroenterol 2013;19(10):1602-10.
33. Taye S, Lakew M. Impact of hepatitis C virus co-infection on HIV patients before and after highly active antiretroviral therapy: an immunological and clinical chemistry observation, Addis Ababa, Ethiopia. BMC Immunol 2013;14:23.
34. Soriano V, Vispo E, Labarga P, Medrano J, Barreiro P. Viral hepatitis and HIV co-infection. Antiviral Res 2010;85(1):303-15.
35. Soriano V, Sheldon J, Garcia-Gasco P, Vispo E. Lack of anti-HIV activity of entecavir in an HIV patient coinfected with hepatitis B and delta viruses. AIDS 2007;21(16):2253-4.
36. W S. Treatment of Chronic Hepatitis C with High Dose Interferon Therapy Experience from Pertamina Central Hospital Jakarta. The Indonesian Journal of Gastroenterology Hepatology and Digestive Endoscopy 2002;3(3):82-85.
37. Nurul Akbar AS, Irsan Hasan, Laurentius Lesmana,Rino Alvani Gan*, Andri Sanityoso, Sjaifoellah Noer,FX Pridady, Soemarno. Efficacy and Safety of In-Asia-manufactured Interferon The Indonesian Journal of Gastroenterology, Hepatology, and Digestive Endoscopy 2009;10(1):7-13.
38. Soriano V, Puoti M, Sulkowski M, Cargnel A, Benhamou Y, Peters M, et al. Care of patients coinfected with HIV and hepatitis C virus: 2007 updated recommendations from the HCV-HIV International Panel. AIDS 2007;21(9):1073-89.

Table 1 Summary of HBsAg and HCV-antibody prevalence among HIV-infected patients in Indonesia

No	Author	Year	Sites	N	population	HBsAg prevalence	HCV-Antibody prevalence
1	Wisaksana *et l.*	2010	Hasan Sadikin hospital, West Java	773	Adult HIV infected patients	7%	71%
2	Nelwan *et al.*	2010	Banceuy Narcotic prison, West Java	46	HIV incoming prisoners	4.9%	89%
3	Anggorowati *et al.*	2012	Dr. Sardjito hospital, Central Java	126	Adult HIV infected patients	3%	32%
4	Utsumi *et al.*	2013	Private clinic, East Java	118	Adult HIV infected patients	15%	Not reported

Chapter 2

Low cost HIV-1 quantitative RT-PCR assay in resource-limited settings: improvement and implementation

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Published in J Virol Methods. 2012 Oct;185(1):118-23

Abstract

Monitoring of HIV viral load in low and middle income settings is limited by high cost of the commercial assays. Therefore, we developed a novel RT-PCR quantitative assay. This assay targets the HIV-1 *pol integrase* gene (INT). Subsequently, performances of the INT assay, a previously described Long Terminal Repeat (LTR) assay and a combined INT/LTR dual target RT-PCR assay were compared. The LTR-assay was found to be sensitive and cost-effective (50-70% cheaper than commercial assays) with the lowest coefficient of variation (%CV). Introduction of an internal standard further improved assay reliability. Therefore, this LTR assay was implemented in West Java, Indonesia. Linearity and precision of the LTR assay were good: %CV ranged from 1.0% to 10.4%. The limit of quantitation was 616 copies/ml. Performance was comparable with the commercial assay (Abbott assay) (r^2= 0.01), although on average the viral loads were 0.39 $\log_{10}$ copies/ml lower. In clinical practice, it had excellent capability for monitoring treatment failure, the positive predictive value was 99% and the negative predictive value was 93%. In conclusion, the implementation of the improved HIV-1 viral load LTR-assay for routine diagnostics in resource poor settings can be a good alternative when commercial assays are unaffordable.

Keywords: *HIV-1 assay, viral load assay, non-subtype B, CRF01_AE*

Introduction

UNAIDS estimates that at the end of 2009, 33.3 million people were living with HIV, 83.6% of them live in low- and middle-income countries.[1] The monitoring of HIV viral load of patients treated with anti retroviral treatment (ART) is an effective and common practice in high-income countries. However, the high cost of the commercial HIV viral load tests limits their implementation in low- and middle-income settings.

Although affordable alternative tests are available for measuring HIV RNA, such as the heat-associated HIV-1 p24 antigen enzyme-linked immunosorbent assay[2-4] and the reverse transcriptase activity kit assay from Cavide Exa Vir[5-8], these have not been implemented widely in low income settings because they are labor-intensive and involve complex techniques. "in-house" real-time quantitative RT-PCR assays have the advantage that they are cheap, sensitive and easy to perform. One of these assays targets the HIV-1 Long Terminal Repeat (LTR), which is moderately conserved among HIV-1 subtypes B and non subtype B. This assay has been evaluated by several research groups and found to have limit of detection varying from 92.4 copies/ml to 300 copies/ml.[9-13] Given the large variation of HIV-1 subtypes found in some developing countries, instead of using single target assay, it could be more advantageous to use dual target assays like the recently described COBAS Taqman HIV-1 v2.0.[14-16]

Most of the "in-house" assays were developed in high-income countries that have adequate laboratory capacity and high skilled technicians, but there are few reports of their implementation in low- or middle-income countries. The difference in capacity between those two settings may affect the technical and clinical performance of the assay. Therefore, it is crucial to evaluate the total performance of the assay in the actual laboratory capacity in resource-limited countries. The assays internal control is important when monitoring the efficiency of extraction and amplification of an individual sample. It is essential to select internal controls that can be mixed in one extraction tube without interfering with the target PCR outcome.

This research was conducted at Erasmus MC, the Netherlands and at Hasan Sadikin hospital, Indonesia. The technical performance of three "in-house" assays, two single (LTR and INT) target assays and one dual target (combination of LTR and INT) assay, in combination with three different nucleic acid extraction procedures were examined. The most optimal configuration

assay was chosen and its implementation in West Java, Indonesia, was evaluated. The ability of the assay to predict therapy failure, and its running cost compare with a commercial HIV-1 viral load assay were assessed. The internal control of the assay was determined and its application with the HIV-1 "in-house" assay was observed.

This study provides information which could be useful for monitoring HIV viral load as a part of a routine service for HIV/AIDS patients under therapy in countries facing financial and capacity constraint.

Material and Methods

Test samples

The WHO HIV-1 RNA standard (HIV-1 RNA, 2nd International Standard, 97/650; NIBSC) containing 5.56 $\log_{10}$ IU of HIV-1, subtype B, per ml was diluted in HIV negative plasma and used to determine the limit of detection (LOD) and the limit of quantitation (LOQ) of each assay. Twenty replicates at each concentration (725, 325, 180, 90 and 45 copies/ml) were extracted using QIAamp RNA viral mini kit using two sample input volumes, 140 µl and 560 µl.

To evaluate the performance of three nucleic acid systems, a HIV-1 culture supernatant was diluted in HIV negative plasma. Ten replicates at concentration 3.29 $\log_{10}$ copies/ml were run per assay per purification system.

To test the efficiency of the assay on different types of the HIV strains, the WHO subtype reference plasma panel (NIBSC code: 08/358,UK) including HIV-1 subtypes A, B, C, D, AE, F, G, H and N was assessed.

To determine the intra- and inter-assay variation, 10 aliquot of HIV-1 IIIB purified virus at concentrations between 7.43 and 2.83 $\log_{10}$ copies/ml were tested. In the assessment of the intra-assay variation, five assays were run independently, while in the inter-assay variation, ten assays were run independently.

A HIV-1 IIIB electron microscopy counted virus stock ($2{,}3x10^{11}$ vp/ml, tebu-bio, France) was calibrated relative to the WHO HIV-1 RNA standard diluted in QIAamp RNA viral mini kit lysis buffer and used as secondary standard and for further experiments.

Clinical samples

To determine the acceptable detection limit of the developed HIV-1 "in-house" assays in resource-limited settings, a total of 2121 clinical samples from 915 patients were tested using the Abbott RealTime HIV-1 assay over a period of four years.

To assess the agreement between "in-house" assay and the Abbott RealTime HIV-1 assay, a total of 210 clinical samples from patients treated with antiretroviral therapy (ART) at Hasan Sadikin Hospital, West Java, Indonesia were selected at random after obtaining informed consent. Those selected samples were also used to define the treatment predictive value of the "in-house" assay. The samples were tested with the Abbott RealTime HIV-1 viral load assay and stored at -80^{0}C until tested by the "in-house" assays. The Bland-Altman analysis, the positive predictive value (PPV) and the negative predictive value (NPV) of the "in-house" assay relatively to the commercial assay were calculated.

RNA isolation

HIV-RNA was extracted with three purification systems: MagnaPure LC (Roche Diagnostics, GmBH), Abbot HIV-1 preparation kit (Abbott Molecular, USA) and QIAamp RNA viral mini kit (Qiagen, GmBH). For the MagnaPure LC system, the input volume was 200 µl and the output volume was 100 µl, for the Abbott HIV-1 system, the input volume was 200 µl and the output volume was 89 µl, for the QIAamp RNA system, the input volume was 140 µl and the output volume was 80 µl. The extraction procedures followed the manufacturer's instructions.

Assay design

Primers and probes for the LTR assay were obtained from Drosten et al. (2006). This assay targets Long Terminal Repeat region of HIV-1. The primers and probe for INT assay were designed targeting conserved HIV-1 *pol* integrase gene (nt 4835-4972) using Primer Express software. To assess the presence of primer/probe mismatches in those two assays, alignments were made using all complete sequences of Los Alamos database. An alignment of the LTR region was built from 257 and of the INT region was built from 1456 different HIV-1 subtype sequences. The INT fragment was amplified using the forward primer 5'-

cataatagcaacagacatacaaactaaaga-3', reverse primer 5'-gccccttcacctttcca-3' and probe 5'-aaaattttcgggtttattacagggacagca-3'. Probes of the LTR and INT assay were labeled with FAM fluorescent dye at the 5' end and non-fluorescent quencher at the 3' end. For the dual target assay (MIX), primers and probes from the LTR and INT assay were combined in a single RT-PCR reaction.

Real-time quantitative RT-PCR

RNA was amplified using the TaqMan® Gold RT-PCR amplification kit (Roche Molecular System, New Jersey, USA). Briefly, 20µl of RNA was added into 30µl amplification-mix containing 20pmol of each primer and 5pmol probe. Thermal cycling program was as follows: 48 °C for 30 min; 95 °C for 10 min; 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Cycling was performed in ABI 7500 sequence detection system (Applied Biosystems, Nieuwkerk a/d Ijssel, the Netherlands). RNAse free water (Baxter, the Netherlands) was used as negative template control (NTC) and low (10^3 copies/ml), mid (10^4 copies/ml) and high (10^6 copies/ml) concentrations of the EM counted virus stock were used as positive external quantitation controls. An obtained Cycle threshold (Ct)-value was valid if the NTC was undetectable and if Ct values of the positive controls were in range of mean ± two times standard deviation (STD).

Internal control

Phocine distemper virus (PDV) was used as a universal internal control (GenBank accession: AF479274). The PDV internal control (IC) was added to each sample upon HIV-1 RNA isolation. PDV RNA was detected in a separate RT-PCR system described by Clancy et al. (2008) and contained forward primer 5'-cgggtgccttttacaagaac-3' and reverse primer 5'-ttctttcctcaacctcgtcc-3' which were modified to generate an 83 base pair amplicon. The probe 5'-atgcaagggccaattcttccaagtt-3' was labeled with a 5'-Dragonfly1 dye and matching 3'quencher BHQ-2. An obtained Ct-value of a sample was valid if the Ct-value of the IC was in range of mean ± two times the standard deviation (STD).

Statistical analysis

Microsoft Office Excel 2003, Sigma Plot for Windows, version 10.0 and SPSS version 15 were used for all statistical analysis described. MedLabQC software version 3.25 (Biologiste des Hopitaux, Metz, France) was used to monitor whether internal and external controls were within the set limits.

Local laboratory capacity

The selected assay was successfully implemented in Hasan Sadikin hospital/public health laboratory, West Java, Indonesia. The laboratory meets the standard molecular diagnostic laboratory requirements. There are five laboratory staff who are responsible for the whole running process from blood sampling, sample procession, qRT-PCR amplification and data analysis.

Results

Assay design and nucleic acid extraction

The percentage of mismatch in primers/probes is presented in Table 1. In the LTR forward and reverse primers, there were 6 out of 257 of HIV-1 strains (2.3%) which had mismatches which in theory could seriously affect RT-PCR amplification. In the INT forward primer mismatches were found in 212 out of 1456 HIV-1 strains (14.6%) while in the INT reverse primer none of the HIV-1 strains had mismatches. However, there were fewer mismatches in LTR (4.4%) and in INT (1.1%) forward primers of HIV-1 CRF01_AE (the HIV-1 strains prevalent in the region where we intended to implement the assay). No PCR affecting mismatches were found in LTR and INT reverse primers of this HIV-1 subtype. Less than two mismatches were found in LTR (4.3%) and INT (0, 0%) probes region of CRF01_AE whereas 4 (17.1%) were found in the LTR probes and 17 (1.2%) in the INT probe. In the analysis of the sequence alignment we could not find evidence for asymmetrical primer binding in the primer regions.

The coefficient of variation and Ct value of each assay are summarized in table 2. Overall, the differences found with the combination of real-time RT-PCR and nucleic acid extraction systems were minor. However, in all methods the LTR assay tended to have the least variation. On the basis of the %CV, cost of nucleic acid extraction per sample and ease of use, the QIAamp RNA viral mini kit was chosen for further study.

LOD and LOQ

The LOD (95% hit rate by probit analysis) and LOQ (10% CV limit) of the different assay formats are given in Table 3. The LOQ of the INT assay could not be determined in both input volumes, because it was above the concentration that we used for this analysis.

Assay selection

In order to choose the optimal detection limit for implementation of the "in-house" assay, a preliminary study was conducted in West Java, Indonesia. A total of 2121 samples from 915 patients were included. Viral load data were obtained using the Abbott HIV-1 amplification kit with a limit of detection 150 copies/ml (Figure 1). Forty-three samples (2.0%) from 43 patients had a viral load above 150 copies/mL but below 600 copies/ml. From 28 of the 43 patients follow-up data could be analyzed. In 24 out of the 28 patients (85.7%) viral load decreased below 150 copies/ml without a change in treatment regimen, while the remaining four (14%) patients had a viral load above 600 copies/ml in their follow-up sample. This analysis showed that the amount of misclassified in defining treatment failure using the detection limit of 600 copies/ml was very low. The cost of the more sensitive 201 copies/ml assay were twice higher (25 USD versus 13 USD) then the 616 copies/ml assay in our setting. We therefore decided on basis of these considerations to study the performance of the in-house assay with a lower limit of 616 copies/ml in the Bandung setting

Figure 2 shows its ability to quantify different HIV-1 genotypes when tested on the WHO HIV-1 subtypes reference panel, with the Abbott RealTime HIV-1 assay as reference. Equal efficiencies between two assays were observed in most of the HIV-1 subtypes. The difference of viral loads greater than 0.5 log10 copies/ml was found only in HIV-1 subtype B (0.64log10 copies/ml).

Linearity and reproducibility of the assay

Figure 3 shows the result of the further experiments in which the linearity and reproducibility of the LTR assay were evaluated in West Java. The intra-assay variability ranged from 0.54% at

7.43 log_{10} copies/ml to 7.51% at 2.83log_{10} copies/ml and the inter-assay variability ranged from 1.02% at 7.43 log_{10} copies/ml to 10.35% at 2.83log_{10} copies/ml (Table 4). The PCR efficiency in both analyses was similar, intra-assay slope was 3.57 and inter-assay slope was -3.77 and both assays had good correlation coefficients (0.98~).

Agreement with Abbott assay

The correlation between the LTR assay and the Abbot RealTime HIV-1 assay using independent clinical samples is shown in Fig. 4. A total of 120 clinical samples that were tested positive by the Abbott assay were evaluated using the LTR assay. Three samples were positive in the Abbott assay but negative in the LTR assay, with the Abbott value of <2.7 log_{10} copies/ml. The agreement between both assays was assessed using the Bland-Altman plot. The slope of the curve was 0.046, and the coefficient correlation (r^2) was 0.01. The mean difference in the plasma HIV-1 RNA levels obtained with the LTR assay and the Abbott assay was -0.39 log_{10} copies/ml (mean 3.9 log_{10} in the LTR assay versus mean 4.3 log_{10} copies/ml in the Abbott assay). At higher viral load ranges, the Abbott assay reported slightly higher values than the LTR assay. The mean differences were 0.29 log_{10} copies/ml at viral loads ranging from 2 to 3.99 log_{10} copies/ml and 0.56 log_{10} copies/ml at viral loads ranging from 5 to 6 log_{10} copies/ml. The proportion of samples under quantified by more than 1 log_{10} with the LTR assay relative to the Abbot Assay was 1.7% (n=2). Reverse transcriptase and protease gene sequences revealed that the outlier patients had HIV-1 subtype CRF01_AE (a common subtype in Indonesia). Reverse transcriptase and protease gene sequences revealed that the outlier patients had HIV-1 subtype CRF01_AE (a common subtype in Indonesia). The LTR fragment sequences of these two patients were 100% matches with the primers and probes targeted by our LTR assay. These data suggest that the underestimation of these HIV-1 viral loads by the LTR assay is largely due to the repeated freeze thawing cycle of these samples. A total of 90 clinical samples that were tested negative by the Abbott assay (<2.18 log_{10} copies/ml) were assessed using the LTR assay. Six samples were negative in the Abbott assay but positive in the LTR assay with value of <2.9 log_{10} copies/ml.

At the virological failure cutoff of 500 copies/ml, the positive predictive value (PPV) was 95% and the negative predictive value (NPV) was 91%. At cutoff of 5000 copies/ml, the PPV was 99% and the NPV was 93% (Table 5).

Discussion

In April 2008, HIV-RNA testing using a commercial assay was introduced at Hasan Sadikin hospital, a referral hospital for ART in West Java, Indonesia. It was part of a program to prevent, control and treatment of HIV infection among drug users.[17] However, the budgetary constrains limit the sustainability of these services. Therefore, an "in-house" assay was developed at the same time and it showed good linearity, reproducibility and good positive and negative values for predicting treatment failure.

One of the crucial factors in developing "in-house" assays is that designed primers and probes can sensitively detect different subtypes of HIV-1. The HIV-1 subtype sequences alignment showed that a low percentage of mismatches affecting RT-PCR efficiency were found in selected primers and probes region. Which suggest that the implementations of both single and dual target assays are feasible for management of HIV-1 infected individual. In settings where only few subtypes circulate the single target assay might be implemented because it is more efficient and cheaper. However, a dual target assay has advantages that it may overcome misinterpretation of the HIV-1 viral load in the settings where higher numbers of different subtypes are circulating (e.g. West African countries). Alternatively, degenerate primers maybe developed to overcome sequence variation in the primer set. This would however need to be properly validated.

In addition, the RNA extraction technique can also influence the PCR outcome. Different extraction procedures can give variability to the result. The presented data showed that the variability of PCR result of the three evaluated assays with automatic and manual RNA extraction techniques was still within a tolerable range. However, the costs of the automatic extraction system, which may limit pipetting errors, are significant in a public health sector in developing countries. Compared to Abbott preparation kit which is based on magnetic beads, the QIAamp

RNA viral mini kit which is based on column was easier to handle and was less labor intensive, therefore in this setting QIAamp viral mini kit was used for further analysis.

To improve patient's clinical management, it is important that an HIV-1 viral load assay has an acceptable detection limit. In order to enhance the sensitivity of the assays described in this paper, two different input volumes, 140 µl and 560 µl, were evaluated. Considering the impact on clinical management and cost accompanying the different assay formats, we decided to implement the assay format with a HIV-1 RNA LOQ of 616 copies/ml. Clearly, the decision on which assay format to select for a particular diagnostic approach, e.g. the one or dual target assay and small or larger input volume will differ per setting and may be influenced by issues such as reimbursement policy, the amount of variation in local circulating subtypes and the level organization of a laboratory.

Managing the linearity and accuracy of a HIV-1 viral load assay is critical in the implementation of "in-house" assays as routine service in resource-limited setting. HIV-1 RNA quantitation in "in-house" developed assays may be influenced by batch to batch variation which is not quality controlled as well as commercial assays. However, when applied in West Java, with two laboratory staff, three different extraction and amplification reagent batches, and during one year of study, the LTR assay showed excellent reproducibility. The intra-assay variability of the LTR assay was comparable to two FDA approved assays. The intra-assay variability at HIV-RNA concentration around 3 $\log_{10}$ copies/ml was 3.31% at the LTR assay, 4.31% at the Roche COBAS® AMPLICOR ™ HIV-1 MONITOR® v1.5 and 1.85% at the Abbott RealTime™HIV-1.[18] The addition of internal and external controls to these assays and the monitoring of these controls over time probably significantly contributed to the stability of HIV-1 RNA quantitation in this setting.

Cost of antiretroviral treatment is a major burden for people living with HIV in many developing countries. One cross-sectional study in two big cities in Indonesia reported that even if the first line ART drugs was delivered free of charge, they still had to spend 68-96% of their monthly expenditure on HIV related care.[19] The LTR "in-house" developed assay has a lower cost than the commercial assays available in Indonesia; the available commercial tests cost were $50-100, while the "in-house" assay cost, including the RNA purification system and qRT-PCR for

internal control, was $12-15. This price can be lowered if multiplex qRT-PCR with internal control or other opportunistic diseases such as HCV or HBV can be developed.

In conclusion, this study provided the evaluation of three different assays and described essential factors that can involve in the PCR outcome, including predictive effect of mismatches in the primers and probes region, variability in the result due to variation of extraction methods, and influence of target volume in determining the detection limit of the assay. Based on variability of the assay, detection limit and cost effectiveness, the LTR assay was implemented in West Java, Indonesia. In this setting, the selected assay showed excellent reproducibility, high correlation with the FDA approved HIV-1 assay, and good predictive value for monitoring treatment failure. The future challenges for this promising virological monitoring is to ensure the reliable sample transfer from rural area to the reference laboratory. Previously, several research groups reported the advantages of dried blood spot in solving the specimen transfer problem for HIV viral load monitoring using a commercial assay and drugs resistance test in rural area. [20-22] Therefore, further research in applying this "in-house" assay with dried blood spot sampling method should be carried on in order to increase the capability of HIV therapy monitoring in resource-limited setting countries.

Acknowledgement

We thank the staff and patients of the Hasan Sadikin Hospital, West Java, Indonesia, for their participation. We are grateful to Dept. of Virology, Erasmus Medical Center, the Netherlands for donating reagents for sample testing and for additional training support in West Java. We acknowledge the financial support of the European Commission (Sixth Framework Programme; Specific Targeted Research Project no: 318／10150A – EC SANTE/2005/105-033).

References

1. UNAIDS. Global report: Unaids report on the global aids epidemic 2010. Switzerland: unaids.org; 2010.
2. Bonard D, Rouet F, Toni TA, Minga A, Huet C, Ekouevi DK, et al. Field evaluation of an improved assay using a heat-dissociated p24 antigen for adults mainly infected with HIV-

1 CRF02_AG strains in Cote d'Ivoire, West Africa. J Acquir Immune Defic Syndr 2003;34(3):267-73.

3. Ribas SG, Ondoa P, Schupbach J, van der Groen G, Fransen K. Performance of a quantitative human immunodeficiency virus type 1 p24 antigen assay on various HIV-1 subtypes for the follow-up of human immunodeficiency type 1 seropositive individuals. J Virol Methods 2003;113(1):29-34.
4. Ataide R, Hasang W, Wilson DW, Beeson JG, Mwapasa V, Molyneux ME, et al. Using an improved phagocytosis assay to evaluate the effect of HIV on specific antibodies to pregnancy-associated malaria. PLoS One 2010;5(5):e10807.
5. Braun J, Plantier JC, Hellot MF, Tuaillon E, Gueudin M, Damond F, et al. A new quantitative HIV load assay based on plasma virion reverse transcriptase activity for the different types, groups and subtypes. AIDS 2003;17(3):331-6.
6. Malmsten A, Shao XW, Aperia K, Corrigan GE, Sandstrom E, Kallander CF, et al. HIV-1 viral load determination based on reverse transcriptase activity recovered from human plasma. J Med Virol 2003;71(3):347-59.
7. Huang D, Zhuang Y, Zhai S, Song Y, Liu Q, Zhao S, et al. HIV reverse transcriptase activity assay: a feasible surrogate for HIV viral load measurement in China. Diagn Microbiol Infect Dis 2010;68(3):208-13.
8. Stewart P, Cachafeiro A, Napravnik S, Eron JJ, Frank I, van der Horst C, et al. Performance characteristics of the Cavidi ExaVir viral load assay and the ultra-sensitive P24 assay relative to the Roche Monitor HIV-1 RNA assay. J Clin Virol 2010;49(3):198-204.
9. Candotti D, Temple J, Owusu-Ofori S, Allain JP. Multiplex real-time quantitative RT-PCR assay for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus type 1. J Virol Methods 2004;118(1):39-47.
10. Drosten C, Panning M, Drexler JF, Hansel F, Pedroso C, Yeats J, et al. Ultrasensitive monitoring of HIV-1 viral load by a low-cost real-time reverse transcription-PCR assay with internal control for the 5' long terminal repeat domain. Clin Chem 2006;52(7):1258-66.
11. Rouet F, Ekouevi DK, Chaix ML, Burgard M, Inwoley A, Tony TD, et al. Transfer and evaluation of an automated, low-cost real-time reverse transcription-PCR test for diagnosis and monitoring of human immunodeficiency virus type 1 infection in a West African resource-limited setting. J Clin Microbiol 2005;43(6):2709-17.
12. Edelmann A, Kalus U, Oltmann A, Stein A, Unbehaun A, Drosten C, et al. Improvement of an ultrasensitive human immunodeficiency virus type 1 real-time reverse transcriptase-polymerase chain reaction targeting the long terminal repeat region. Transfusion 2010;50(3):685-92.

13. Vessiere A, Rousset D, Kfutwah A, Leoz M, Depatureaux A, Simon F, et al. Diagnosis and monitoring of HIV-1 group O-infected patients in Cameroun. J Acquir Immune Defic Syndr 2010;53(1):107-10.
14. Paba P, Fabeni L, Ciccozzi M, Perno CF, Ciotti M. Performance evaluation of the COBAS/TaqMan HIV-1 v2.0 in HIV-1 positive patients with low viral load: a comparative study. J Virol Methods 2011;173(2):399-402.
15. Sire JM, Vray M, Merzouk M, Plantier JC, Pavie J, Maylin S, et al. Comparative RNA quantification of HIV-1 group M and non-M with the Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 v2.0 and Abbott Real-Time HIV-1 PCR assays. J Acquir Immune Defic Syndr 2011;56(3):239-43.
16. Sizmann D, Glaubitz J, Simon CO, Goedel S, Buergisser P, Drogan D, et al. Improved HIV-1 RNA quantitation by COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0 using a novel dual-target approach. J Clin Virol 2010;49(1):41-6.
17. Alisjahbana B, Susanto H, Roesli R, Yusuf H, Hinduan Z, Mase JC, et al. Prevention, control and treatment of HIV-AIDS among injecting drug use in Bandung, Indonesia. Acta Med Indones 2009;41 Suppl 1:65-9.
18. Crump JA, Scott LE, Msuya E, Morrissey AB, Kimaro EE, Shao JF, et al. Evaluation of the Abbott m2000rt RealTime HIV-1 assay with manual sample preparation compared with the ROCHE COBAS AmpliPrep/AMPLICOR HIV-1 MONITOR v1.5 using specimens from East Africa. J Virol Methods 2009;162(1-2):218-22.
19. Riyarto S, Hidayat B, Johns B, Probandari A, Mahendradhata Y, Utarini A, et al. The financial burden of HIV care, including antiretroviral therapy, on patients in three sites in Indonesia. Health Policy Plan 2010;25(4):272-82.
20. Charpentier C, Gody JC, Tisserand P, Matta M, Fournier J, Mbitikon O, et al. Usefulness of a genotypic resistance test using dried blood spot specimens in African HIV-infected children with virological failure according to the 2010-revised WHO criteria. Arch Virol 2011;156(9):1603-6.
21. Bertagnolio S, Parkin NT, Jordan M, Brooks J, Garcia-Lerma JG. Dried blood spots for HIV-1 drug resistance and viral load testing: A review of current knowledge and WHO efforts for global HIV drug resistance surveillance. AIDS Rev 2010;12(4):195-208.
22. Johannessen A. Dried blood spots in HIV monitoring: applications in resource-limited settings. Bioanalysis 2010;2(11):1893-908.
23. Stadhouders R, Pas SD, Anber J, Voermans J, Mes TH, Schutten M. The effect of primer-template mismatches on the detection and quantification of nucleic acids using the 5' nuclease assay. J Mol Diagn 2010;12(1):109-17.

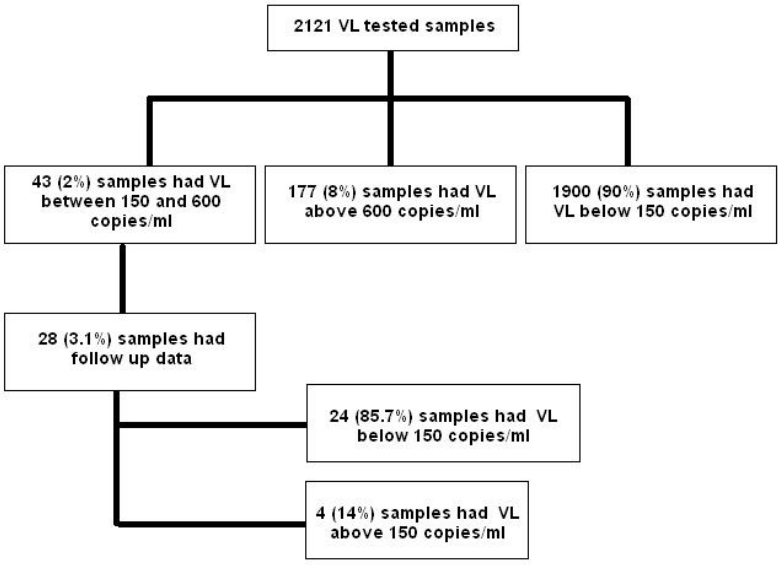
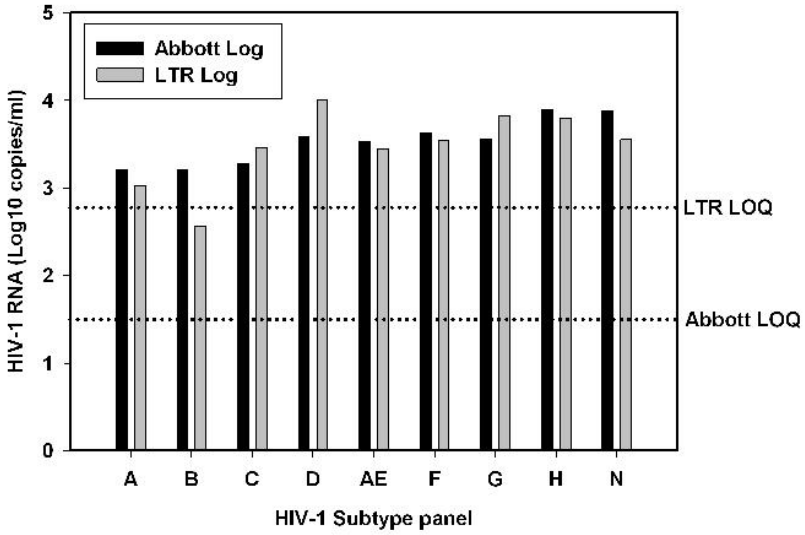


Figure 1 Investigation scheme of the true treatment failure patients study in the West Java cohort



Log10 different	0.18	0.64	0.19	0.41	0.09	0.07	0.27	0.1	0.33

Figure 2 WHO HIV-1 subtype reference panels tested using the Abbott and LTR assays

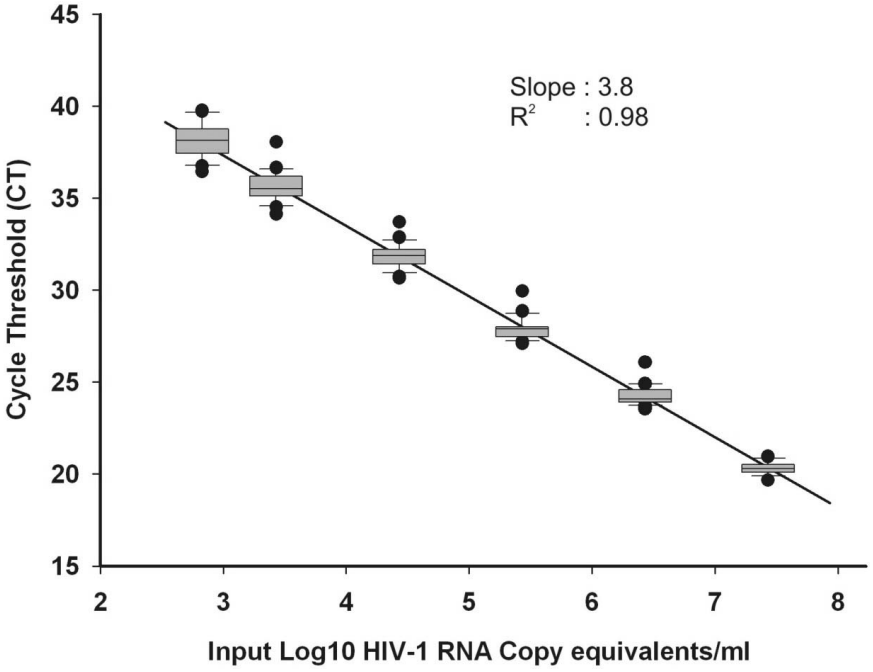


Figure 3 The linearity of the HIV-1 LTR real-time PCR assay in West Java, Indonesia.

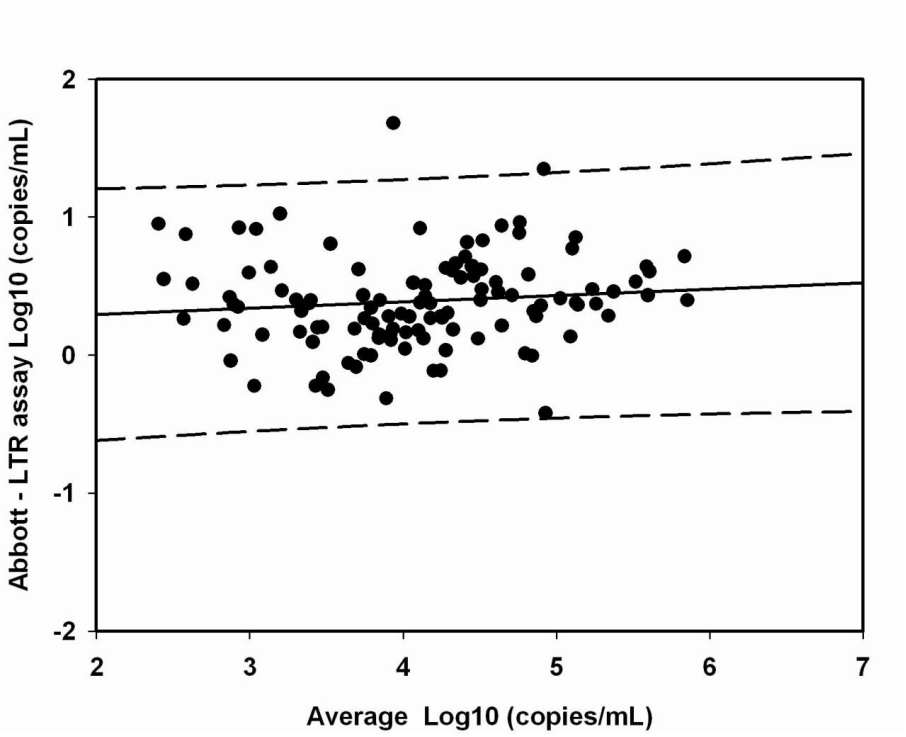


Figure 4 Comparison results of the Abbott HIV-1 and LTR "in-house" assay on clinical samples. Regression plot based on Bland Altman analysis-derived data from the Abbott HIV-1 assay compared with the result for the HIV-1 LTR assay in Bandung, Indonesia (r= 0.01; N=117). Viral loads measured were $\log_{10}$ transformed before analysis. Dashed lines indicate 1 $\log_{10}$ difference from the regression line

Table 1 Mismatch in the LTR and INT primers and probes. The alignments were derived from different HIV-1 subtypes; 257 subtypes in the LTR and 1456 subtypes in the INT

Primers/probes (length)	Number of strains that have at least one mismatch (%)	Number of strains that have more than two mismatches (%)	Number of strains that have mismatches affecting RT-PCR efficiency (%)*
LTR Forward (24 bp)	41 (15.9)	32 (12.5)	6 (2.3)
LTR Reverse (19 bp)	15 (5.8)	2 (0.78)	6 (2.3)
LTR Probe (26 bp)	32 (12.5)	4 (1.5)	-
INT Forward (30 bp)	780 (53.6)	84 (5.8)	212 (14.6)
INT Reverse (16 bp)	97 (6.7)	2 (0.14)	0 (0)
INT Probes (30 bp)	715 (49.1)	17 (1.2)	-

*Stadhouders et al., 2010[23]

Table 2 Variation of RT-PCR result in three nucleic acid purification systems

Purification system	Mean Ct value/%CV		
	INT assay	LTR assay	Mix INT-LTR assay
QIAamp viral RNA mini kit	30.31/10.86	33.37/5.71	32.42/8.47
Abbott HIV-1sample preparation kit	36.22/13.74	31.05/3.78	32.45/10.69
MagnaPure LC	34.73/6.55	31.99/6.48	32.54/9.6

Table 3 Limit of detection (LOD) and limit of quantification (LOQ) of the single and dual target assays

In-house assay	volume samples (µl)	LOD* (copies/ml)	LOQ** (copies/ml)
INT	140	1248	-
	560	424	-
LTR	140	450	616
	560	184	201
MIX INT-LTR	140	755	800
	560	266	305

* LOD is defined as the 95% hit rate by probit analysis
** LOQ is defined as the concentration where the 10% CV is exceeded

Table 4 Intra- and inter-assay accuracy of the LTR assay

No. of copies	Intra assay %CV	Inter-assay %CV
$2.69.\ 10^7$	0.54	1.02
$2.69.\ 10^6$	0.95	1.67
$2.69.\ 10^5$	0.46	2.62
$2.69.\ 10^4$	1.18	3.71
$2.69.\ 10^3$	3.32	6.56
$6.67.\ 10^2$	7.51	10.35

Table 5 Positive and negative predictive values of the LTR assay relative to the Abbott assay with two different virological failures cutoff values

Virological failure cutoff value (copies/ml)	No. of true positive samples[a]	No. of false positive samples[b]	No. of true negative samples[c]	No. of false negative samples[d]	No. of total samples[e]	Positive predictive value (%)	Negative predictive value (%)
500	109	6	84	8	207	95	91
5000	77	10	119	1	207	99	93

[a] Number of samples that have HIV RNA concentration above virological failure cutoff when tested by both LTR and Abbott assays
[b] Number of samples that have HIV RNA concentration above virological faiure cutoff when tested by the LTR assay but have HIV RNA concentration below virological failure cutoff when tested by the Abbott assay
[c] Number of samples that have HIV RNA concentration below virological failure cutoff when tested by both LTR assays and Abbott assay
[d] Number of samples that have HIV RNA concentration below virological failure cutoff when tested by the LTR assay but have HIV RNA concentration above virological failure cutoff when tested by the Abbott assay
[e] Number of samples tested by the LTR and Abbott assay

Chapter 3

Virological failure and drug resistance during first line anti-retroviral treatment in Indonesia

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Published in J Med Virol. 2013 Aug;85(8):1394-401

Abstract

We studied virological response and development of drug resistance during first-line anti-retroviral treatment (ART) in Indonesia where the majority of HIV-infected patients have a history of injecting drug use, which is often linked with lower treatment adherence and development of drug-resistance. We prospectively followed 575 patients starting ART between September 2007 and March 2010 in Hasan Sadikin Hospital Bandung. Clinical and laboratory monitoring was performed every 6 months. Plasma samples with HIV-RNA ≥400 copies /ml were examined for drug resistance mutations. Most patients were male (72.3%), 59.7% had a history of injecting drug use, and the median CD4 cell count before start of ART was 35 cells/mm^3 (IQR 10-104). From 438 HIV patients with HIV-RNA measurements, 40 (9.1%) subjects had HIV-RNA ≥400 copies/ml after 24 weeks (median follow-up 16 (IQR 8-25) months). Of these failing patients 16 (47%) subjects had drug resistance mutations, predominantly M184V (35.3%), Y181C (23.5%), K103N (11.7%) and TAMs (11.7%). A history of treatment discontinuation ≥1 month, reported by 5.3% (23) of patients, was strongly associated with virological failure (adjusted OR 12.64, 95% CI 4.51-35.41); and a history of IDU was not (OR 0.75, 95% CI 0.38-1.46). This is the largest and most systematic evaluation of virological response to first line ART in Indonesia. Patients in this cohort responded well to first line ART, with low rates of virological failure and drug resistance. A history of injecting drug use should not be a reason to withhold ART in this setting.

Keywords: *Antiretroviral therapy; Asia; HIV drug Resistance; Viral load*

Introduction

The development of antiretroviral treatment has dramatically reduced morbidity and mortality among HIV-infected individuals, and has turned HIV infection into a chronic condition for many patients. The success of HIV treatment depends on maintaining long-term viral suppression, which also avoids development of HIV resistance to anti-retroviral drugs. Unfortunately, HIV-RNA plasma measurements and drug resistance testing are often not generally available in low- or medium income countries. As a result, reliable data about effectiveness of first and second line ART in those settings are often lacking. This is also true for Indonesia, which has one of the most rapidly growing HIV epidemics in Asia. HIV-RNA measurements can only be performed in a limited number of institutes, mostly in the capital Jakarta and not routinely for all patients. As a result, viral resistance may accumulate and spread, but so far this has not been analyzed systematically.

The Indonesian epidemic is different from many other epidemics, as it is a concentrated epidemic largely driven by injecting drug use, except for Papua which has a more generalized epidemic. The HIV prevalence in the general population is around 0.2%, but among injecting drug users (IDUs) 50% or more are found HIV-seropositive. [1, 2]Many IDUs have not been tested and for those who are HIV-infected, as few as 6% are currently getting HIV-treatment.[1] Health providers tend to think that IDUs have a low adherence to treatment [3] , and thus are at risk to develop and spread drug resistant HIV.

We therefore conducted a prospective cohort study in an urban setting in West-Java to examine the rate of virological failure, possible risk factors for failure and the development of drug resistance. Almost two-thirds of patients of our cohort are infected through injecting drug use allowing us to examine injecting drug use as a risk factor for failure of ART and development of drug resistant viruses.

Methods

Setting and study population

This study was embedded in a five-year program called 'IMPACT', aiming to prevent, control and treat HIV among injecting drug users (IDUs) in West-Java, Indonesia.[4] At the time of the study,

IMPACT was supporting patient care in three clinics in Bandung, the capital of West Java (40 million people): a teaching hospital, a methadone clinic, and a prison clinic. In these clinics, people with and without a history of injecting drug use, who are at risk for HIV infection or who present with signs and symptoms suggesting HIV/AIDS are counseled and tested for HIV infection. All testing is voluntary and informed consent is obtained from all study participants. From all patients baseline data are collected including: socio-demographics, medical history, injecting and sexual risk behavior, physical examination and plasma HIV RNA and CD4 cell counts. HIV-infected patients are characterized and followed prospectively in a cohort study, which has been approved by the Health Research Ethics Committee at the Faculty of Medicine of Padjadjaran University/Dr. Hasan Sadikin General Hospital in Bandung, Indonesia.

First-line ART, consisting of efavirenz (EFV) or nevirapine (NVP) in combination with lamivudine (3TC), and stavudine (d4T) or zidovudine (ZDV), is provided free of charge. Since 2007, tenofovir (TDF) and ritonavir-boosted lopinavir (LPV/r) are available as second line ART for a limited number of HIV-infected patients failing first-line ART. At the time of the study, following 2006 World Health Organization (WHO) guidelines, ART was indicated for HIV-infected patients presenting with CD4 < 200 cells/mm^3 or with WHO clinical stage III or IV. All patients receive adherence counseling before starting ART.

After initiation of ART, patients return to the clinic monthly for medication, with CD4 and HIV-RNA testing done usually every six months. CD4 cell counts are examined using Facscount flow cytometry technology (BD Biosciences, Jakarta Indonesia), and HIV-RNA is quantified by real-time polymerase chain reaction (RT-PCR) (Abbot, IL, USA) with a detection limit of 150 copies HIV-RNA/mm^3. Virological failure for this analysis was defined as HIV RNA more than 400 copies/ml after at least 24 weeks therapy. However, patients were switched to second-line ART if HIV-RNA > 10^4 copies/ml were detected after at least six months ART despite reporting sufficient treatment adherence.[5]

Treatment adherence is evaluated each visit and every six months, using a systematic assessment of self-reported treatment discontinuations $\geq$ 1 month and missed ART doses in the last three days, last week and last month. Patients considered having suboptimal adherence or possible treatment failure receive more intensive counseling. For this study, the level of

adherence (or average adherence for patients with multiple assessments) was calculated, and good adherence was defined as ≥ 95% reported medication intake. Factors that can affect adherence and response to ART, e.g. disclosure of HIV-status to the spouse or close relatives, living conditions, concurrent TB treatment and other issues, were explored using standardized interviews during baseline and follow-up. For this study blood samples from virological failure patients were sent to the Erasmus Medical Center in Rotterdam, The Netherlands, for genotypic drug resistance testing. The reverse transcriptase and protease genes from failure patients were amplified using in-house primers [6] and analyzed by Sanger sequencing method.

Data analysis and statistics

We retrieved data from all adult patients who started ART between September 2007 and March 2010. The primary end-point during follow-up was HIV-RNA > 400 copies/ml after a minimum of six months of ART, measured at one or more time points. Data are presented as mean [standard deviation (SD)] if distributed normally, median [interquartile range (IQR)] if not distributed normally, or as a proportions. Differences between groups were examined using chi-square test for proportions and Mann-Whitney test for continuous variables. Predictors of virological failure were examined using logistic regression and expressed as odds ratios (OR) with 95% confidence interval (CI), factors with $p<0.25$ in univariate analysis were included in multivariate analysis[7]. All statistical analysis was performed using SPSS version 16.0.1. The genes sequences were processed using DNASTAR Lasergene 8 USA, bioinformatics software. HIV sub typing was determined using REGA HIV-1 sutyping tool Version 2.0 (HIVdb, Stanford University). Drug resistance mutations were determined using HIVdb program, genotypic resistance interpretation algorithm (Stanford University). Potential second line drugs options were defined based on drug resistance interpretations and mutations scores (HIVdb, Stanford University).

Results

Patient characteristics

A total of 595 consecutive HIV patients who started their ART between September 2007 and March 2010 were included. The majority were young men with a history of injecting drug use and advanced HIV infection with very low CD4 cell counts before start of ART (**Table 1**). Female patients, many diagnosed as a result of partner notification, had higher CD4 cell counts (median 76 cells/mm^3, IQR 23-183) compared to men (median 27 cells/mm^3, IQR 9-73) (p=0.001). Compared to men, women less frequently reported a history of injecting drug use (18.4% vs. 80.5%, p<0.001), as also seen in a lower prevalence of HCV infection (27.6% vs. 79.6%, p<0.001). Both male and female patients were well educated, 87.5% had completed secondary school, probably because in the '90s drug use typically started among the higher socioeconomic class in Indonesia. The large majority (93.9%, 559) of HIV infections was diagnosed in the hospital, while 3.5% (21) of patients were diagnosed in the methadone clinic (MMT)[8] and 2.5% (15) in prison[9] as part of targeted testing among high-risk groups. HIV patients who started ART in MMT and prison had slightly higher CD4 cell counts (median 95 cells /mm^3, IQR 20-165) compared to HIV patients in hospital (median 33 cells /mm^3, IQR 10-95) (p=0.01).

Treatment response

During follow-up of a median 16 (IQR 8-25) months, 66 patients (11.1%) died, the large majority (48; 73%) within the first six months. In addition, 60 patients (10.1%) were lost to follow-up, most (42; 70%) within six months. Patients who died had a lower CD4 cell count (median 23 cells /mm^3, IQR 6-49) compared to those lost to follow-up (median 37 /mm^3, IQR 5-104). Another 21 patients (3.5%) were transferred, while in 10 patients (1.6%) no HIV-RNA measurement was done (**Figure 1**). HIV-RNA was examined in 438 patients who had taken $\geq$ 6 months ART, with a median of 1.7 measurements per patient. Virological failure (HIV RNA range 2.7 – 6.2 $\log_{10}$ copies/ml) was found in 40 patients (9.1 %). The median time from start of first line ART until detection of failure was 11 (range 6-31) months, and within this group 25% (10) had undetectable plasma HIV-RNA before being diagnosed with virological failure. These patients showed low-level HIV-RNA plasma concentrations (< 3 $\log_{10}$ copies/ml) during ART in 12.5% (5), while 30.0% (12) had 3-4 $\log_{10}$ copies/ml, 22.5% (9) had 4-5 $\log_{10}$ copies/ml and 35.0% (14) had > 5 $\log_{10}$ copies HIV-RNA/ml. Viral load measurements were repeated in 24 patients after a median of 7 (range 4-19) months;

in 15 (62.5%) patients virological failure was observed. Adherence counseling revealed that nine patients were not taking their ART at the time of HIV-RNA measurement, four of them had >5 log_{10} copies HIV-RNA/ml.

CD4 cell measurements following start of ART, available for 78.0 % (464) of HIV patients, showed a favorable immunological response in most patients. The median CD4 cell count increased from 35 (10-104) cells/mm^3 before start of ART to 163 (IQR 103-224) cells/mm^3 after 6 months of therapy, 211 (IQR 151-279) cells/mm^3 after 12 months, 254 (IQR 197-382) cells/mm^3 after 18 months, and 340 (IQR 245-369) cells/mm^3 after 24 months ART. In line with previous studies, WHO immunological failure criteria [WHO, 2006] had a low positive predictive value (PPV) of 25.8% for virological failure, and a reasonable negative predictive value (PPV) (92.5%), the latter because of the low occurrence of virological failure.

Risk factors for virological failure

The single most important factor associated with detectable plasma HIV RNA was a history of treatment discontinuation $\geq$ 1 month (**Table 2**). Among 23 patients who reported an interruption of ART, 11 (47.8%) were diagnosed with detectable HIV, compared with 29 of 415 patients (7%) who had had no treatment interruption. Reasons for treatment discontinuation could be specified for 17 out of 23 patients, with side effects in six patients and inability to collect medication because of travel out of town as the main reasons for five patients. Four patients mentioned relapse of drug use as the main reason, one patient mentioned he “forgot” his medication, and another patient felt he had to take too many drugs.

In univariate analysis, patients who were young, unmarried or living alone, and those who had used NVP instead of EFV also seemed at higher risk for virological failure, but these associations lost statistical significance in multivariate regression analysis. Interestingly, injecting drug use, present in almost two thirds in this population, was not associated with virological failure. Similarly, low CD4 cell count, male gender, low education, unemployment, lack of disclosure of HIV status to spouse or family, distance from the hospital, nor concurrent TB treatment during start of ART showed an association with failure (**Table 2)**.

Drug resistance mutations

Genotypic analysis was performed on samples obtained from 34 out of 40 failure patients (For six patients not enough material was available). Based on REGA HIV-1 subtyping approach, all analyzed samples were HIV CRF01_AE except two of them. One sample was HIV-1 subtype A1, and the other one was unidentified. Drug resistances mutations were found in 16 (47%) patients (**Table 3**). The M184V mutation (confining resistance to lamivudine) was seen in 12 out of 16 patients, additional NRTI mutations were present in 7 out of 16 patients. Thymidine Associated Mutations (TAMs), associated with resistance to stavudine and zidovudine, were found in four patients. The K65R mutation (resistance to tenofovir) occurred in two patients, both together with M184V and NNRTI mutations. The most common NNRTI mutation was Y181C (n=8) followed by K103N (n=4). Four patients had resistance to all three antiretroviral drugs they were taken, whereas 8 patients showed resistance to two drugs in their regimens. Protease resistance associated mutations were not found.

For all patients (n=16) with drug resistance mutations the sensitivity to drugs available in Indonesia was evaluated using the Stanford scoring system. Twelve patients harbored virus that showed resistance to 3TC, four to zidovudine, seven for stavudine and three to tenofovir. In fourteen out of 16 patients, HIV isolates were resistant to the first generation NNRTI (efavirenz and nevirapine).

A comparison between failing patients with and without drug resistance mutations revealed that patients in whom no HIV drug resistance mutations were detected had higher HIV RNA plasma levels (Median 5.1 (3.5-5.7) $\log_{10}$ copies/ml *vs* 4.0 (3.8-5) $\log_{10}$ copies/ml p= 0.11) and lower CD4 counts (Median 63 (20-197) cells/mm^3 *vs* 199 (125-299) cells/mm^3 p= 0.05). This suggests that resistance associated mutations may not have been detected because the patients were not adherent. There was no relevant association between NVP failure and development of drug resistance mutations (p= 0.68).

Discussion

To our best knowledge, this is the first study from Indonesia reporting combined data of HIV plasma RNA measurements and drug resistance testing in patients starting first line ART. Based

on intention to treat analysis, the occurrence of virological failure was lower compared to those obtained in a multicenter cohort study in the Asia-Pacific region[10] and to results from a systematic review including 89 cohort studies in Africa [Barth et al., 2010]. The low occurrence of failure might suggest that all patients starting ART in Indonesia have a good prognosis. Unfortunately, early mortality in our patients was very high and in line with earlier studies from India [9] and Indonesia.[10] Early loss to follow-up was also high, and as most patients in this group presented with advanced disease (average CD4: 37 cells/mm^3) it is likely that many have died as well. This has been reported in other low-resource settings: in a study performed in Malawi, 50% of patients registered as 'lost to follow-up' were actually found to have died during home visits.[11] Clearly, earlier detection of HIV infection is a high priority for Indonesia, like for many other low-resource settings.

It is well-known that non-adherence to ART leads to virological failure.[12-15] In our study, long periods of ART discontinuation (for more than one month) constituted the single most important risk factor for virological failure. Similar findings have been reported from studies in Africa, Europe and America. [16-18] Interestingly, incomplete drug intake (suboptimal adherence) was not associated with virological failure in our setting. The adherence threshold of 95% that we used was based on early studies using the first generation NNRTI and more complex proteas inhibitor (PI)-based regimens. However, currently used regimens may be more 'forgiving'. [19, 20] The use of NVP as opposed to EFV seemed a risk factor for virological failure, although this association was not significant in multivariate analysis (OR 2.02, p=0.06). This difference has also been reported by studies from India and Africa[21, 22], but caution is warranted as the prescribed regimens may have been subject to indication bias.

Injecting drug use was not a risk factor for virological failure during ART. In contrast to what is often stated, treatment adherence did not significantly differ between IDUs and non-IDUs, and treatment discontinuations (4.5% respectively 4.4%) were similar. A systematic review about injecting drug use and adherence to ART found that IDUs on average display lower adherence to treatment, but that similar adherence levels can be achieved compared to non-IDUs by comprehensive care, management of co-morbidities, psychosocial support and particularly by opioid substitution.[23]

Monitoring response to ART in resources limited setting is challenging. In line with previous studies we found that WHO immunological failure criteria lack sensitivity and specificity when compared to virological criteria.[24] Unfortunately, measurement of plasma HIV-RNA is costly, and routine HIV-RNA monitoring is generally beyond the financial capacity of low-resource settings. Mathematical modeling using data from Africa has shown that viral load testing did not have an impact on long-term survival[25], and this was recently confirmed in a large clinical trial. [26] Plasma HIV-RNA measurement might however impact drug resistance development; a meta-analysis has shown that patients treated in settings without viral load monitoring had significantly more resistance mutations, compared to patients in settings where plasma HIV-RNA is measured every three months.[27] In our study, the M184V mutation and and NNRTI resistance mutations were most common. The availability of virological monitoring in our setting probably limited the development of TAMs (present in only 4 out of 34 patients), since the development of these mutations take longer periods of virological failure. This finding is in agreement with other reports from low-income countries where virological monitoring is part of routine care.[28]

In our study, no drug resistance mutations were found in more than half of the patients with virological failure. Most likely this is a result of very low compliance, with low drug exposure and a low risk of development of drug resistance mutations as a result. However, in the absence of longitudinal resistance data and given the fact that our genotyping was performed using population sequencing, we cannot rule out that resistant viruses were selected in these patients but were not detected in plasma at the time of failure.[29] This may be a particular problem for low genetic barrier drugs such as the first generation NNRTI. [30]In that case continuing / restarting patients on their first line regimen poses a risk of further selection of more resistant viruses.

We observed that the absolute value of their HIV-RNA concentration could help to select those who needed switching to second-line ART. Patients with very high plasma HIV-RNA levels (> $5\log_{10}$ copies/ml) and very low CD4 counts (<100 cells/mm^3) during ART (probably as a result of prolonged treatment interruptions) were less likely to harbor drug resistant virus compared to those with viral 'blips' or low HIV-RNA concentrations (< $3\log_{10}$ copies/ml) who were probably taking ART, but not with full adherence.

Our data indicate that the currently available second line antiretroviral drugs in Indonesia, tenofovir and ritonavir-boosted lopinavir, are probably effective, supporting the current WHO recommendation for second-line ART.[31] Thirteen out of 16 patients who developed resistance during first line therapy were still considered to be sensitive to tenofovir, based on population sequencing. Furthermore, no resistance was found against protease inhibitors. However, no clinical trial has evaluated the effectiveness of different second line regimens in resource limited settings. Potential options for second line are either to recycle drugs from the first line, or to use second line options. Whereas recycling first line drugs is attractive given the lower costs, it also has the potential risk of reduced drug activity due to selection of resistant viruses.[32] Genotyping may help to minimize this problem, although the absence of mutations does not exclude the possibility of clinically relevant minority species. Constituting a new regimen based on boosted protease inhibitors (PI) seems attractive given the high genetic barrier of these drugs. Further studies are required to determine whether additional drugs are needed and which additional drugs should be added. Particularly the role of 3TC/FTC in a setting where the M184V is selected has not been resolved. A previous report has shown however that continuation of lamivudine is beneficial regarding immunological response and clinical progression in patients with limited second lines options.[33] Of interest, In the absence of PI-mutations, ritonavir boosted lopinavir monotherapy proved effective for patients with extensive resistance to NRTIs and NNRTI in a cohort study in Thailand with three year follow-up.[34]

Our study had several limitations. Plasma HIV-RNA measurements were not available for all patients, injecting drug use and adherence data were derived from self-reporting, and adherence evaluation was limited to the last month of therapy. Despite these limitations, our study clearly demonstrates that patients in our setting in Indonesia have a good virological response to first line ART consisting of a NNRTI combined with 3TC and an additional nucleoside inhibitor. Importantly injecting drug use, often considered a risk factor for non-adherence and treatment failure, although very common in this setting was not associated with poor outcome. This indicates that a history of injecting drug use should not be a reason to withhold ART. Identifying HIV patients at risk of non-adherence is important, as they may be target for intensive adherence interventions.[6, 35] At present, regular viral load measurements cannot be done in

Indonesia. Future studies should help design cost-effective monitoring strategies, as was done in other settings.[36, 37] In addition, efforts are underway to implement cheaper 'in-house' viral load assays.

Acknowledgments

We thank all study participants. Nirmala Kesumah, Mery Lestari, Ajeng Pratiwi, Intan Meilana, Ega Rogayah, Nuni Haeruni, Panji Anugerah, Lendi Kostaman and Irma Utama helped collecting data and maintaining the HIV cohort. This study was supported by 'IMPACT' (Integrated Management of Prevention And Care and Treatment of HIV/AIDS), a collaborative research and implementation program of Padjadjaran University, Bandung, Indonesia; Maastricht University and Radboud University Nijmegen, the Netherlands; and Antwerpen University, Belgium. IMPACT is funded by the European Commission (SANTE/2005/105-033). R.W. has a fellowship from the Radboud University; B.A. has a post-doc fellowship from the Royal Dutch Academy of Arts and Sciences (KNAW); R.vC. has a VIDI grant from the Netherlands Foundation of Scientific Research (NWO).

R.W., A.F., D.vdV., M.S., B.A., P.S., C.B., A.vdV., and R.vC. designed the study. R.W., A.F., D.vdV., and R.vC. performed statistical analysis. R.W. and A.F. wrote the first draft of the article. All authors contributed to the interpretation of the results and to the final version of the manuscript. R.W., A.F., A.I., and Y.H. were involved in data acquisition and data management. R.W. and A.F. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosure statement

None of the authors declares any conflict of interest.

References

1. National AIDS comission. Republic Indonesia country report on the follow up to the declarrations of commitment on HIV/AIDS (UNGASS): Reporting period 2008-2009; 2009.

2. Iskandar S, Basar D, Hidayat T, Siregar IM, Pinxten L, van Crevel R, et al. High risk behavior for HIV transmission among former injecting drug users: a survey from Indonesia. BMC Public Health 2010;10:472.
3. Werb D, Wood E, Small W, Strathdee S, Li K, Montaner J, et al. Effects of police confiscation of illicit drugs and syringes among injection drug users in Vancouver. Int J Drug Policy 2008;19(4):332-8.
4. Alisjahbana B, Susanto H, Roesli R, Yusuf H, Hinduan Z, Mase JC, et al. Prevention, control and treatment of HIV-AIDS among injecting drug use in Bandung, Indonesia. Acta Med Indones 2009;41 Suppl 1:65-9.
5. WHO. Antiviral therapy for HIV infection in adults and adolescents in resource limited settings; towards universal access 2006.
6. Hart JE, Jeon CY, Ivers LC, Behforouz HL, Caldas A, Drobac PC, et al. Effect of directly observed therapy for highly active antiretroviral therapy on virologic, immunologic, and adherence outcomes: a meta-analysis and systematic review. J Acquir Immune Defic Syndr 2010;54(2):167-79.
7. Hosmer DW LS, editor. Applied logistic regression. New York: Wiley; 1989.
8. Achmad YM, Istiqomah AN, Iskandar S, Wisaksana R, van Crevel R, Hidayat T. Integration of methadone maintenance treatment and HIV care for injecting drug users: a cohort study in Bandung, Indonesia. Acta Med Indones 2009;41 Suppl 1:23-7.
9. Nelwan EJ, Van Crevel R, Alisjahbana B, Indrati AK, Dwiyana RF, Nuralam N, et al. Human immunodeficiency virus, hepatitis B and hepatitis C in an Indonesian prison: prevalence, risk factors and implications of HIV screening. Trop Med Int Health 2010;15(12):1491-8.
10. Oyomopito R, Lee MP, Phanuphak P, Lim PL, Ditangco R, Zhou J, et al. Measures of site resourcing predict virologic suppression, immunologic response and HIV disease progression following highly active antiretroviral therapy (HAART) in the TREAT Asia HIV Observational Database (TAHOD). HIV Med 2010;11(8):519-29.
11. Yu JK, Chen SC, Wang KY, Chang CS, Makombe SD, Schouten EJ, et al. True outcomes for patients on antiretroviral therapy who are "lost to follow-up" in Malawi. Bull World Health Organ 2007;85(7):550-4.
12. Ekstrand ML, Shet A, Chandy S, Singh G, Shamsundar R, Madhavan V, et al. Suboptimal adherence associated with virological failure and resistance mutations to first-line highly active antiretroviral therapy (HAART) in Bangalore, India. Int Health 2011;3(1):27-34.
13. Jordan MR, La H, Nguyen HD, Sheehan H, Lien TT, Duong DV, et al. Correlates of HIV-1 viral suppression in a cohort of HIV-positive drug users receiving antiretroviral therapy in Hanoi, Vietnam. Int J STD AIDS 2009;20(6):418-22.

14. Maneesriwongul WL, Tulathong S, Fennie KP, Williams AB. Adherence to antiretroviral medication among HIV-positive patients in Thailand. J Acquir Immune Defic Syndr 2006;43 Suppl 1:S119-22.
15. Shah B, Walshe L, Saple DG, Mehta SH, Ramnani JP, Kharkar RD, et al. Adherence to antiretroviral therapy and virologic suppression among HIV-infected persons receiving care in private clinics in Mumbai, India. Clin Infect Dis 2007;44(9):1235-44.
16. Ncaca LN, Kranzer K, Orrell C. Treatment interruption and variation in tablet taking behaviour result in viral failure: a case-control study from Cape Town, South Africa. PLoS One 2011;6(8):e23088.
17. Parienti JJ, Das-Douglas M, Massari V, Guzman D, Deeks SG, Verdon R, et al. Not all missed doses are the same: sustained NNRTI treatment interruptions predict HIV rebound at low-to-moderate adherence levels. PLoS One 2008;3(7):e2783.
18. Rougemont M, Stoll BE, Elia N, Ngang P. Antiretroviral treatment adherence and its determinants in Sub-Saharan Africa: a prospective study at Yaounde Central Hospital, Cameroon. AIDS Res Ther 2009;6:21.
19. Maggiolo F, Ravasio L, Ripamonti D, Gregis G, Quinzan G, Arici C, et al. Similar adherence rates favor different virologic outcomes for patients treated with nonnucleoside analogues or protease inhibitors. Clin Infect Dis 2005;40(1):158-63.
20. Paterson DL, Swindells S, Mohr J, Brester M, Vergis EN, Squier C, et al. Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. Ann Intern Med 2000;133(1):21-30.
21. Datay MI, Boulle A, Mant D, Yudkin P. Associations with virologic treatment failure in adults on antiretroviral therapy in South Africa. J Acquir Immune Defic Syndr 2010;54(5):489-95.
22. Kamya MR, Mayanja-Kizza H, Kambugu A, Bakeera-Kitaka S, Semitala F, Mwebaze-Songa P, et al. Predictors of long-term viral failure among ugandan children and adults treated with antiretroviral therapy. J Acquir Immune Defic Syndr 2007;46(2):187-93.
23. Malta M, Strathdee SA, Magnanini MM, Bastos FI. Adherence to antiretroviral therapy for human immunodeficiency virus/acquired immune deficiency syndrome among drug users: a systematic review. Addiction 2008;103(8):1242-57.
24. Boyd MA. Current and future management of treatment failure in low- and middle-income countries. Curr Opin HIV AIDS 2010;5(1):83-9.
25. Phillips AN, Pillay D, Miners AH, Bennett DE, Gilks CF, Lundgren JD. Outcomes from monitoring of patients on antiretroviral therapy in resource-limited settings with viral load, CD4 cell count, or clinical observation alone: a computer simulation model. Lancet 2008;371(9622):1443-51.

26. Lynen L, Van Griensven J, Elliott J. Monitoring for treatment failure in patients on first-line antiretroviral treatment in resource-constrained settings. Curr Opin HIV AIDS 2010;5(1):1-5.
27. Gupta RK, Hill A, Sawyer AW, Cozzi-Lepri A, von Wyl V, Yerly S, et al. Virological monitoring and resistance to first-line highly active antiretroviral therapy in adults infected with HIV-1 treated under WHO guidelines: a systematic review and meta-analysis. Lancet Infect Dis 2009;9(7):409-17.
28. Gallant JE, DeJesus E, Arribas JR, Pozniak AL, Gazzard B, Campo RE, et al. Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. N Engl J Med 2006;354(3):251-60.
29. Metzner KJ, Giulieri SG, Knoepfel SA, Rauch P, Burgisser P, Yerly S, et al. Minority quasispecies of drug-resistant HIV-1 that lead to early therapy failure in treatment-naive and -adherent patients. Clin Infect Dis 2009;48(2):239-47.
30. Li JZ, Paredes R, Ribaudo HJ, Svarovskaia ES, Metzner KJ, Kozal MJ, et al. Low-frequency HIV-1 drug resistance mutations and risk of NNRTI-based antiretroviral treatment failure: a systematic review and pooled analysis. JAMA 2011;305(13):1327-35.
31. WHO. Antiretroviral therapy for HIV infection in adults and adolescents: recommendation for public health approach:2010 revision; 2010.
32. Sigaloff KC, Ramatsebe T, Viana R, de Wit TF, Wallis CL, Stevens WS. Accumulation of HIV drug resistance mutations in patients failing first-line antiretroviral treatment in South Africa. AIDS Res Hum Retroviruses 2012;28(2):171-5.
33. Castagna A, Danise A, Menzo S, Galli L, Gianotti N, Carini E, et al. Lamivudine monotherapy in HIV-1-infected patients harbouring a lamivudine-resistant virus: a randomized pilot study (E-184V study). AIDS 2006;20(6):795-803.
34. Manosuthi W, Kiertiburanakul S, Amornnimit W, Prasithsirikul W, Thongyen S, Nilkamhang S, et al. Treatment outcomes and plasma level of ritonavir-boosted lopinavir monotherapy among HIV-infected patients who had NRTI and NNRTI failure. AIDS Res Ther 2009;6:30.
35. Nachega JB, Mills EJ, Schechter M. Antiretroviral therapy adherence and retention in care in middle-income and low-income countries: current status of knowledge and research priorities. Curr Opin HIV AIDS 2010;5(1):70-7.
36. Abouyannis M, Menten J, Kiragga A, Lynen L, Robertson G, Castelnuovo B, et al. Development and validation of systems for rational use of viral load testing in adults receiving first-line ART in sub-Saharan Africa. AIDS 2011;25(13):1627-35.
37. Lynen L, An S, Koole O, Thai S, Ros S, De Munter P, et al. An algorithm to optimize viral load testing in HIV-positive patients with suspected first-line antiretroviral therapy failure in Cambodia. J Acquir Immune Defic Syndr 2009;52(1):40-8.

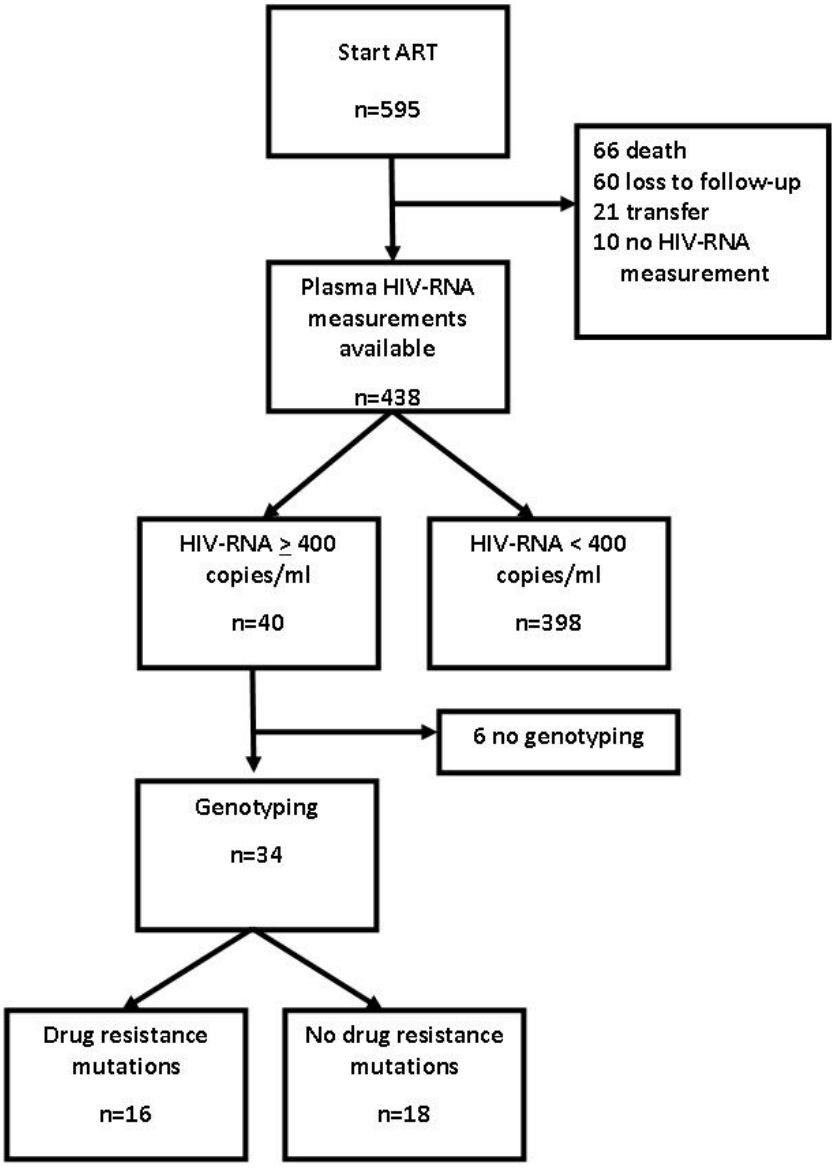

Figure 1 Patients flow

Table 1 Baseline characteristics of patients taking ART (n=595)

Characteristics	
Mean age (SD), years	30 (6)
Male gender, %	72.3
Reported mode of HIV transmission, %	
Injecting drug use	59.7
Heterosexual	34.1
Unknown	6.2
Education, %	
Elementary school	3.8
Primary high school	8.6
Secondary high school	51.6
Academy, University	35.9
Employment, %	
Employed / self-employed	60.2
Not employed	39.8
Marital status, %	
Unmarried	34.5
Married	51.4
Divorced / widowed	14.1
Median CD4 (IQR), cells/mm3	35 (10-104)
Stage WHO, %	
Stage 1	14.5
Stage 2	6.0
Stage 3	27.9
Stage 4	51.6
Receiving TB treatment, %	20.7
HBsAg positive, %	7.1
Anti HCV positive, %	64.9
ART regimen at initiation	
AZT/3TC/NVP, %	45.0
AZT/3TC/EFV, %	22.5
d4T/3TC/NVP, %	15.1
d4T/3TC/EFV, %	16.6
Other, %	0.7

[a]Data were missing for age (n=5), education (n=47), working status (n=25), marital status (n=41), CD4 (n=93), HBsAg (n=47), Anti HCV (n=56), prior ART use (n=58)

Table 2 Factors associated with virological failure (n=438)

	VL≥ 400 copies/ml n=40	VL< 400 copies/ml n=398	Univariate		Multivariate	
			OR (95% CI)	*P-value*	OR (95% CI)	*P-value*
Socio-demographics						
Age ≤ 30 years old, %	77.5	65.2	1.83 (0.85-3.97)	0.12	1.66 (0.72-3.80)	0.23
Male gender, %	77.5	73.9	1.22 (0.56-2.65)	0.62		
IDU as main mode of HIV transmission, %	60.0	66.8	0.75 (0.38-1.46)	0.39		
Low education, %	15.8	10.6	1.58 (0.62-4.02)	0.34		
Not working, %	38.5	38.8	0.99 (0.50-1.94)	0.97		
Unmarried, %	44.7	32.9	1.65 (0.84-3.25)	0.15	1.46 (0.69-3.08)	0.69
Living alone, %	17.5	7.3	2.68 (1.09-6.58)	0.03	2.19 (0.81-5.88)	0.12
No disclosure of HIV status, %	15.4	13.7	1.14 (0.46-2.87)	0.78		
Living outside the city, %	22.5	28.9	0.71 (0.33-1.55)	0.39		
Clinical status						
WHO stage 3-4 at initiation, %	75.8	76.5	0.96 (0.42-2.21)	0.92		
CD4 ≤ 50 cells/mm^3 at initiation, %	55.9	55.2	1.03 (0.51-2.09)	0.94		
Receiving TB treatment, %	12.5	20.6	0.55 (0.21-1.45)	0.55		
ART regimen						
Initial NNRTI used, %						
NVP	72.5	59.5	1.79 (0.87-3.69)	0.11		
EFV	27.5	39.7	0.58 (0.28-1.19)	0.14		
Type of NNRTI last used, %						
NVP	60.0	44.7	1.85 (0.96-3.60)	0.07		
EFV	40.0	51.8	0.62 (0.32-1.21)	0.16		
Always on NVP	60.0	43.7	1.93 (0.99-3.75)	0.05	2.02 (0.96-4.23)	0.06
On initial regimen, %	77.5	68.3	1.60 (0.74-3.45)	0.24		
ART, %						
Adherence < 95%	3.1	3.1	1.02 (0.13-8.12)	0.99		
Treatment discontinuation ≥ 1 month	30.0	2.8	15.08 (6.11-37.22)	<0.001	12.92 (4.57-36.56)	<0.001
Medication collected by others	3.4	2.6	1.33 (0.16-10.87)	0.79		

[a]Data were missing for age (n=1), education (n=32), working status (n=15), marital status (n=29), living condition (n=3), disclosure status (n=42), city address (n=11), WHO stage (n=81), CD4 (n=69), HBsAg (n=16), Anti HCV (n=25), prior ART use (n=39), adherence (n=49), collection of medication (n=65).

Table 3 Drug resistance mutations (n=16)

Patients ID	CD4 (cells/mm^3)	HIV-RNA (log_{10}copies/ml)	ART duration (months)	Initial regimen (months)	Second regimen (months)	NRTI mutations		NNRTI mutations
						3TC	ZDV/d4T	
1	269	3.8	13	3TC/ZDV/NVP (1)	3TC/d4T/NVP (12)	M184V	K65R	Y181C
2	598	3.1	11	3TC/ZDV/NVP (1)	3TC/d4T/EFV (10)	M184V	-	K103N
3	284	4.0	15	3TC/ZDV/NVP (15)		M184V	K65R	Y181C, V108I
4	250	4.1	12	3TC/ZDV/NVP (12)		M184V	M41L[b], T215F[b]	Y181C, A98G
5	177	4.6	12	3TC/ZDV/NVP (12)		M184V	-	G190A
6	66	5.4	11	3TC/ZDV/NVP (11)		M184V	D67N[b], K70R[b],K219E[b]	Y181C
7	393	3.9	7	3TC/ZDV/NVP (1)	3TC/ZDV/EFV (6)	M184V	-	K103N
8	163	4.7	6	3TC/d4T/EFV (6)		M184V	-	-
9	112	3.9	7	3TC/ZDV/NVP (7)		M184V	-	Y181C
10	406	3.8	7	3TC/ZDV/NVP (7)		M184V	-	Y181C
11	304	3.4	10	3TC/ZDV/EFV (10)		M184V	K70N[c]	K103N
12	169	5.6	14	3TC/ZDV/NVP (14)		-	-	Y181C
13	8	5.1	6	3TC/ZDV/NVP (6)		-	K70R[b]	Y181C, K101E, G190A
14	167	2.7	11	3TC/d4T/EFV (8)	3TC/ZDV/EFV (3)	-	K219E[b]	-
15	11	5.2	14	3TC/ZDV/EFV (14)		-	-	Y188L
16	222	4.2	7	3TC/ZDV/NVP (7)		M184V	-	K103N

[a]If applicable; [b]TAMs; [c]Unusual mutation at this position

Chapter 4

Increased risk of virological failure with lamivudine compared to emtricitabine in tenofovir and nevirapine containing initial antiretroviral regimens for therapy-naive HIV-1 infected patients

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Submitted

Abstract

Objectives Lamivudine and emtricitabine are equally recommended in antiretroviral regimens for HIV-1, with lamivudine available as generic formulation. The effectiveness of lamivudine compared to emtricitabine is unclear. The aim of this study was to compare virological responses of lamivudine with emtricitabine, both with tenofovir/nevirapine in once daily regimens, within 12 months of initiating treatment.

Methods Prospective observational cohort study was conducted between 2002 and 2012, included therapy-naive HIV-1 infected adults without baseline resistance. Virological failure (VF) (HIV-RNA >400 copies/mL) within 12 months of initiating therapy were compared using univariate analysis, multivariate logistic regression and survival analyses. Acquired resistance to reverse transcriptase was evaluated.

Results 196 Patients were included for on-treatment analysis. 26 Of 86 patients on lamivudine (30.2%) and 17 of 110 patients on emtricitabine (15.5%) experienced VF (P=0.01). Lamivudine was independently associated with VF (odds ratio: 2.19, 95%CI: 1.05-4.59), shorter time to VF (Log-Rank: P=0.009) and an increased hazard ratio on VF (2.01, 95%CI: 1.09-3.70), adjusted for baseline CD4 <200 cells/mm^3 and HIV-RNA ≥100.000 copies/mL. HIV-RNA at VF was 1 log higher for lamivudine compared to emtricitabine (median 34,650 copies/mL vs. 3,470 copies/mL, P=0.014). Patients experiencing VF on lamivudine acquired more resistance to reverse transcriptase compared to emtricitabine (92.0% vs. 62.5%, P=0.02). 7 Patients on emtricitabine had HIV-RNA >400 (median 558) copies/mL at 12 months without evidence of acquired resistance and achieved suppression (<50 copies/mL) without changing therapy.

Conclusions: The use of lamivudine/tenofovir/nevirapine was associated with lower virological responses compared to emtricitabine/tenofovir/nevirapine as initial antiretroviral regimen for therapy-naive HIV-1 patients.

Keywords: *HIV-1, antiretroviral therapy, virological failure, drug resistance.*

Introduction

Guidelines on initial antiretroviral regimens for therapy-naive HIV-1 infected patients exist for resource-limited and resource-rich settings and promote the use of a non-nucleoside reverse transcriptase inhibitor (non-NRTI) in combination with a NRTI backbone.[1, 2] These guidelines include lamivudine and emtricitabine as equally recommended NRTI in combination with tenofovir and nevirapine containing regimens. Lamivudine has recently become available as generic NRTI in resource-rich countries. An increased use of generic lamivudine to replace branded emtricitabine for cost-effectiveness reasons can be expected.[3]

However, the relative antiretroviral effectiveness of lamivudine compared to emtricitabine has been unclear since in vitro studies observed a potential decreased potency of lamivudine against HIV-1.[4-7] These observations were followed by clinical trials suggesting inferior virological responses of certain lamivudine containing antiretroviral regimens [8-16] with increased rates of acquired drug resistance.[17-19] None of these clinical trials exclusively compared the treatment effectiveness of lamivudine and emtricitabine due to the use of fixed dose co-formulated tablets. The observed virological responses could therefore be significantly confounded by the NRTI co-formulations in the comparator arms instead of an intrinsic lower potency of lamivudine. Furthermore, the treatment responses of lamivudine and emtricitabine in specifically tenofovir and nevirapine containing regimens have only been evaluated in one retrospective study, which was conducted in a resource-limited setting.[20, 21] In contrast to the studies that compared lamivudine and emtricitabine in various co-formulations, this study showed no significant difference between these specific regimens.

As lamivudine and emtricitabine in combination with tenofovir and nevirapine are recommended and widely used antiretroviral regimens, more data on the relative antiretroviral potency of lamivudine versus emtricitabine in tenofovir and nevirapine containing regimens are necessary. The aim of this observational cohort study was to compare the virological

responses of lamivudine with emtricitabine, each in combination with tenofovir and nevirapine, as once daily prescribed treatment regimens for therapy-naive HIV-1 infected patients without baseline resistance.

Methods

Study population

All HIV-1 infected adults ≥18 years of age, initiating either lamivudine/tenofovir/nevirapine or emtricitabine/tenofovir/nevirapine between August 2002 and January 2012 at the Erasmus University Medical Center in Rotterdam were included in the study cohort. The demographical, clinical and virological data were prospectively collected by the Dutch HIV monitoring foundation, which includes the large majority (>95%) of HIV patients from all HIV treatment centers in the Netherlands. All patients gave informed consent for inclusion in this cohort and collection of the data. Included patients were scheduled at least once half-yearly for clinical assessment and measurement of plasma HIV-RNA. Longitudinal measurements of HIV-RNA, reasons for switching regimens, second-line antiretroviral regimens, biochemical and clinical adverse events were collected during follow up. Baseline data on age, sex, origin, route of HIV-1 transmission, CD4 cell count, HIV-RNA levels before the start of antiretroviral therapy and previous antiretroviral exposure were collected at the inclusion in the study cohort. Both treatment regimens were prescribed as once daily regimens during the entire study period. Antiretroviral experienced patients were excluded from the study cohort as were patients with baseline resistance to (non-)NRTI if the resistance was of at least of intermediate resistance, according to the Stanford HIV database algorithm [22].

Study design

The primary endpoint were the proportions of HIV-1 patients with virological failure (VF) during the first year of treatment with lamivudine/tenofovir/nevirapine compared to treatment with

emtricitabine/tenofovir/nevirapine as initial antiretroviral regimen. VF was defined as (1) an unsuccessfully suppressed HIV-RNA <400 copies/mL at the 12 months time-point or (2) a HIV-RNA >400 copies/mL between the time-points 1 and 12 months which was interpreted and documented by the treating physician as VF and resulted in a switch of antiretroviral regimen. A detectable HIV-RNA >400 copies/mL at the 12 months time-point preceded and followed by HIV-RNA <50 copies/mL was considered as a viral blip, not as VF. The HIV-RNA measurement which was used for the 12 months time-point had to be available within a ≥10 and ≤14 months timeframe from the start of the antiretroviral regimen. The most approximate HIV-RNA to the 12 months time-point within this timeframe was used.

We followed an on-treatment analysis of the primary endpoint, including patients that completed treatment with the included regimens from baseline to the 12 months time-point. Patients that were lost to follow up, died or switched their antiretroviral regimens for other reasons than VF were excluded from this analysis. Exclusion followed if these events occurred before the 12 months time-point, given that these patients had not experienced VF previously.

Sensitivity analyses were performed to investigate the robustness of the observed results. We followed an intention to treat analysis including all patients of the cohort to see whether the exclusion of patients from the on-treatment analysis were biasing the results. In this analysis, discontinuation of antiretroviral therapy for any reason was considered equal to VF. A second sensitivity analysis was performed to evaluate the persistence of the observed difference in VF after the 12 months time-point until the most recent documented follow up or until a patient switched regimen for other reasons than VF.

We evaluated second line antiretroviral regimens and sequenced HIV-1 reverse transcriptase of patients at VF to evaluate acquired genotypic HIV-1 resistance of at least intermediate resistance to (non-)NRTI. No genotypic sequencing was done on HIV-RNA <1,000 copies/mL. All plasma samples were stored at -80 °C. For HIV genotyping, the reverse transcriptase gene was amplified using in-house primers.[23] All amplified products were

analyzed by the Sanger method. Resistance mutations were determined using the HIVseq program, genotypic resistance interpretation algorithm.[22]

Statistical analysis

The data are described as mean with standard deviation or median with interquartile range, depending on distribution. Continuous data were compared using the independent T-test or Mann-Whitney U test, if appropriate. The Chi^2 test was used to compare categorical data. Univariate logistic regression was used to compare the proportions of VF between the treatment groups. A multivariate logistic regression model on VF was constructed including the covariates antiretroviral regimen, baseline HIV-RNA (<100,000 or ≥100,000), baseline CD4 cell count (<200 or ≥200) and all other baseline variables that had an association with VF (defined as a P ≤0.1) in univariate analysis. Time to VF was analysed by using Kaplan Meier curves. Patients without VF during follow up that died, were lost to follow up before the 12 months time-point or who changed regimens for reasons other than VF were right censored. A Cox proportional hazards analysis was used to test the effect of explanatory variables on the hazard for VF. The incidences of acquired drug resistance mutations at the time of VF were described. We prespecified a two sided P value at 0.05 to reject null hypotheses.

Results

Baseline characteristics of the study population

Between August 2002 and January 2012, 254 adult HIV-1 patients initiated either once daily lamivudine/tenofovir/nevirapine (n= 112) or once daily emtricitabine/tenofovir/nevirapine (n= 142). Inclusion requirements for the study cohort were not satisfied in 7 patients due to the previous use of antiretroviral drugs in 3 patients, an unavailable baseline genotyping in 1 patient and baseline resistance in 3 patients. The baseline resistance patterns included 2 patients with K103N mutation and 1 patient with multiple thymidine analogue mutations. In total

247 patients (111 on lamivudine, 136 on emtricitabine) were included in the study cohort. 51 Patients were excluded for the on-treatment analysis; side effects caused 27 patients (11 on lamivudine, 16 on emtricitabine) to change their regimens, 5 patients in each treatment group were lost to follow up, 11 patients (7 on lamivudine, 4 on emtricitabine) died without VF and 3 patients (2 on lamivudine) had other reasons to discontinue their antiretroviral regimens prior to the 12 months time-point. The final study cohort for the analysis included 196 HIV-1 infected patients.

The baseline characteristics of the patients are shown in Table 1. Lamivudine in combination with tenofovir/nevirapine was initiated in 86 patients and emtricitabine in combination with tenofovir/nevirapine was initiated in 110 patients as initial antiretroviral regimens. Both treatment groups consisted predominantly of male patients (77.9% and 77.3%), born in resource-rich countries and the average age was 41 years in both groups. Men having sex with men accounted for HIV-1 transmission in 48% of patients on lamivudine and 60% of patients on emtricitabine. The median baseline HIV-RNA was 94,900 copies/mL for patients initiating lamivudine compared to 57,050 copies/mL for patients initiating emtricitabine (P= 0.014). No significant differences were observed in the proportions of patients with baseline HIV-RNA ≥100,000 copies/mL (47.7% compared to 35.5%, P= 0.104). The median baseline CD4 cell count was significantly lower (P= 0.02) in the lamivudine group (200 cells/mm^3) compared to the emtricitabine group (225 cells/mm^3). The proportions of patients with a CD4 cell count <200 cells/mm^3 were comparable between groups (48.8% compared to 40.9%, P= 0.116). The median starting year of patients initiating lamivudine/tenofovir/nevirapine was 2004 (interquartile range (IQR): 2003 -2005). No patient initiated lamivudine/tenofovir/nevirapine after 2008. Baseline characteristics of the excluded patients were comparable (all P >0.05) between the lamivudine and emtricitabine groups (data not shown).

Evaluation of virological responses

The proportion of VF was twofold higher in patients that initiated lamivudine/tenfovir/nevirapine (30.2%) compared to patients that initiated emtricitabine/tenofovir/nevirapine (15.5%, P= 0.01, Table 2). The odds ratio (OR) on VF for a patient initiating lamivudine was 2.37 (95% confidence interval (95%CI): 1.19 - 4.74, P= 0.015) compared to emtricitabine in univariate analysis. The observed differences in VF were confirmed by the sensitivity analyses. The results were consistent in the intention to treat analysis of VF (P= 0.02, supplementary data) and in the analysis of the proportions of VF until the most recent documented follow up (P= 0.01, supplementary data). Considerably higher proportions of patients on both lamivudine (P= 0.013) and emtricitabine (P=0.001) experienced VF if baseline CD4 cell counts were <200 cells/mm^3. A baseline HIV-RNA ≥100,000 copies/mL was associated with VF for both the lamivudine and the emtricitabine containing regimens (P= 0.03 and P= 0.028 respectively). No other baseline variables were associated with VF in the univariate analysis (P >0.1 for all).

The results of the multivariate logistic regression model are shown in Table 3 and provide the adjusted OR on VF for the antiretroviral regimens, corrected for baseline CD4 cell count (<200 versus ≥200 cells/mm^3) and baseline HIV-RNA (<100,000 versus ≥100,000 copies/mL). The use of lamivudine/tenofovir/nevirapine was independently associated with VF (OR 2.19, 95%CI: 1.05 - 4.59) compared to the use of emtricitabine/tenofovir/nevirapine as initial antiretroviral regimen (P= 0.037). The Kaplan Meier curves on time to VF for each regimen are shown in Figure 1. Patients that initiated treatment with lamivudine experienced VF earlier compared to emtricitabine (Log-Rank test: P= 0.009). The Cox proportional hazards analysis showed that the use of lamivudine remained independently associated with an increased hazard for VF (hazard ratio: 2.01, 95%CI: 1.09 - 3.70, P= 0.026) compared to emtricitabine, adjusted for a baseline CD4 cell count <200 cells/mm^3 and HIV-RNA ≥100,000.

All patients that experienced VF had genotypic resistance testing and the results are shown in Table 4. One genotype was unavailable for resistance testing in each treatment group. At the 12 months time-point, 10 of the 16 failing patients (62.5%) in the emtricitabine

group had acquired at least one resistance mutation to reverse transcriptase compared to 23 of the 25 failing patients (92.0%) in the lamivudine group (P= 0.02). The most prevalent acquired resistance mutations to NRTI In both treatment groups were K65R and M184V/I. Y181C/I were the most frequent acquired resistance mutations to non-NRTI. V106A, Y188C and G190A resistance mutations were only observed in patients failing lamivudine and K101E/Q mutations were only observed in patients failing emtricitabine. The majority (80%) of patients with evidence of resistance in the lamivudine treatment group and all patients with evidence of resistance in the emtricitabine treatment group had multiple resistance mutations to reverse transcriptase.

The median HIV-RNA of patients experiencing VF on emtricitabine/tenofovir/nevirapine (3,470 copies/mL, IQR: 622 - 46,100) was 1 log lower than in patients experiencing VF on lamivudine/tenofovir/nevirapine (34,650 copies/mL, IQR: 7,815 - 92,400, P= 0.014). Seven patients on emtricitabine had a HIV-RNA >400 copies/mL at the 12 months time-point (median 558 copies/mL, IQR: 420 - 1350). These patients did not have evidence of acquired genotypic resistance or genotyping was not successful as HIV-RNA were below 1000 copies/ml. All 7 patients were counseled on therapy compliance and continued their initial emtricitabine regimen. This resulted in HIV-RNA <50 copies/mL within 9 months in all patients, sustained until the most recent documented follow up (median 1,379 days, IQR: 1,165 - 1,398). A boosted protease inhibitor based second line regimen was initiated in the 10 other patients on emtricitabine with evidence of acquired resistance and in 24 patients on lamivudine at VF. 1 Patient on lamivudine was switched to etravirine based second line therapy and 1 patient on lamivudine with an acquired M184V mutation at VF did not switch to a second line regimen immediately per patient's request.

Discussion

This study compared the virological responses of lamivudine with emtricitabine, both combined with tenofovir and nevirapine in once daily regimens, as initial antiretroviral therapy for HIV-1 infected patients without baseline resistance. The use of lamivudine/tenofovir/nevirapine was associated with more virological failure within the first year of treatment compared to the use of emtricitabine/tenofovir/nevirapine. Furthermore, the use of lamivudine in combination with tenofovir/nevirapine resulted in a shorter time to VF, a higher HIV-RNA at VF and a higher prevalence of acquired drug resistance to reverse transcriptase compared to the use of emtricitabine with tenofovir/nevirapine. Of notice, 7 patients on emtricitabine had low level viraemia (median HIV-RNA <1000 copies/mL) without evidence of acquired resistance at the 12 months time-point. Although considered as VF per study protocol, their initial antiretroviral regimens were continued which resulted in successful virological suppression (HIV-RNA <50 copies/mL) after adherence counselling.

Our study has several strengths and the results could have several implications. The observed differences in VF and the successful continuation of emtricitabine/tenofovir /nevirapine in HIV-1 patients with low level viraemia might reflect a higher genetic barrier of emtricitabine to acquired drug resistance. The possible biological explanation for these observations could be the longer intracellular half-life, the favourable median effective concentration and the more efficient incorporation in HIV-DNA by reverse transcriptase of emtricitabine compared to lamivudine.[4, 5, 7, 24] Adherence counselling and continuation of emtricitabine/tenofovir/nevirapine in HIV-1 patients with low detectable HIV-RNA, without evidence of genotypic resistance, may be a valid option instead of switching to more expensive and possible more toxic second line therapies.

This study is the first and largest to specifically compare the virological responses of lamivudine with emtricitabine in tenofovir/nevirapine containing once daily antiretroviral regimens in a resource-rich setting. The only study that directly compared these regimens observed a non-significant 5% difference in VF.[20] However, this study was conducted

retrospectively in a resource-limited setting with VF defined as HIV-RNA above 1,000 copies/mL. Furthermore, the available clinical trials are confounded by the use of co-formulated drugs in the regimens.[8-16] Our results confirm the observations by 2 small pilot studies of 59 patients in total on lamivudine combined with tenofovir and nevirapine. One of these studies was uncontrolled and the other did not include emtricitabine in the control group.[25, 26] These pilot studies observed VF in 25% and 30% of patients on lamivudine/tenofovir/nevirapine respectively. Interestingly, VF occurred predominantly in patients with high baseline HIV-RNA (>100,000 copies/mL) and both studies were prematurely discontinued.

An important possible implication for future guidelines is raised. Lamivudine and emtricitabine are currently recommended as equals for the use in tenofovir/nevirapine containing regimens and other recommended regimens. The generic availability of lamivudine could favour the use of generic lamivudine over branded emtricitabine for cost-effectiveness and cost-containment in HIV-1 care. Our study highlights that an increased use of lamivudine may significantly hamper treatment effectiveness in certain regimens. A twofold increase of VF on generic lamivudine with subsequent more expensive second line therapies and monitoring could exceed initial savings. Decreased virological responses on lamivudine could also support ongoing HIV transmission on population level. These issues should be subject of future studies before a switch from initiating emtricitabine to initiating lamivudine in therapy-naive patients is advocated for cost-effectiveness.

Several limitations of this study should be noticed. First and most importantly, we cannot rule out the possibility of unmeasured confounders due to the consecutive treatment of HIV-1 patients over time. Therapy guidelines have changed over the 10 year study period, especially on the decision when to start antiretroviral therapy.[27] Patients included earlier in the cohort initiated antiretroviral therapy at later stages of the disease than the current standard of care. Although the median baseline CD4 cell count was significantly lower and the median baseline HIV-RNA was significantly higher in the lamivudine treatment group, the absolute

differences were small (25 CD4 cells/mm^3 and 0.5log HIV-RNA copies/mL). Moreover, the proportion of patients in the most unfavorable baseline CD4 cell count and HIV-RNA subgroups (CD4 <200 cells/mm^3 and HIV-RNA ≥100.000 copies/mL) did not differ significantly. The multivariate logistic regression model was used for adjustment of these confounding factors. Second, medication adherence may have been different between regimens; patients on the once daily lamivudine containing regimen took 1 pill extra compared to patients on the once daily emtricitabine containing regimen, due to the availability of coformulated emtricitabine/tenofovir. It is in our opinion unlikely that this difference could explain a twofold increase in VF on lamivudine. Furthermore, the size of the cohort limited further categorization of patients in order to identify high-risk subgroups for VF on lamivudine. Finally, this observed difference in VF might not apply to patients once their HIV-RNA has declined below a certain threshold. If equally effective, a switch from emtricitabine to lamivudine once HIV-RNA is below 400 copies/ml, could save costs without loss of virological effectiveness. Future studies should assess these questions and randomized clinical trials are needed to confirm our observed differences in virological response between lamivudine and emtricitabine.

We specified a HIV-RNA of 400 copies/mL as cut off point for VF, while modern HIV-RNA assays are increasingly sensitive using cut off points as low as 20 copies/mL. Although a viraemia between 20 - 50 copies/mL has been shown to be associated with viral rebounds,[28] it remains unclear whether this low level viraemia influences the rates of VF.[29] It is in fact the persistence of a sustained HIV-RNA above 400 copies/mL that has been identified to increase mortality.[30] By defining VF as a HIV-RNA above 400 copies/mL, we intended to add specificity and clinical relevance to the study for best approximation of clinical practice.

In conclusion, lamivudine and emtricitabine are equally recommended by guidelines in combination with tenofovir and nevirapine as part of initial antiretroviral regimens for the treatment of therapy-naive HIV-1 infected patients. The availability of generic lamivudine is likely to increase the use of lamivudine over emtricitabine for cost-saving purposes. This study

suggests that during the first year of treatment, the use of emtricitabine results in improved virological responses compared to lamivudine in tenofovir/nevirapine containing regimens. In our opinion, this substantial and clinically relevant effect on treatment effectiveness outweighs the increased costs at least during the first year of antiretroviral therapy.

Acknowledgements

Casper Rokx and Bart J.A. Rijnders designed the study and collected the data. Azzania Fibriani and Martin Schutten collected and analyzed the genotypic resistance patterns. Casper Rokx, Bart J.A. Rijnders and David A.M.C. van de Vijver analyzed the data. Casper Rokx and Bart J.A. Rijnders wrote the first version of the manuscript. Annelies Verbon critically reviewed the final version of the manuscript. The results of this study have been presented in part at the 14th European AIDS Conference in Brussels (October 17th 2013, Abstract PS4/4). Bart J.A. Rijnders reports a research grant from Gilead Sciences outside the context of the submitted work. David A.M.C. van de Vijver acknowledges support from the European Union funded project Collaborative HIV and Anti-HIV Drug Resistance Network (CHAIN) outside the context of the submitted work. None of the authors has any other potential conflict of interest.

References

1. World Health Organization. Consolidated Guidelines on the use of Antiretroviral Drugs for Treating and Preventing HIV Infection. Page 112. Available at: http://apps.who.int/iris/bitstream/10665/85321/1/9789241505727_eng.pdf. Accessed on: October 13, 2013. In.
2. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. Section F-4, Table 5.2a, page 74. Available at: http://aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf. Accessed on: August 30, 2013. In.

3. Walensky RP, Sax PE, Nakamura YM, Weinstein MC, Pei PP, Freedberg KA, et al. Economic savings versus health losses: the cost-effectiveness of generic antiretroviral therapy in the United States. Ann Intern Med 2013;158(2):84-92.
4. Feng JY, Anderson KS. Mechanistic studies comparing the incorporation of (+) and (-) isomers of 3TCTP by HIV-1 reverse transcriptase. Biochemistry 1999;38(1):55-63.
5. Schinazi RF. Assessment of the relative potency of emtricitabine and lamivudine. J Acquir Immune Defic Syndr 2003;34(2):243-5; author reply 245-6.
6. Moore KH, Barrett JE, Shaw S, Pakes GE, Churchus R, Kapoor A, et al. The pharmacokinetics of lamivudine phosphorylation in peripheral blood mononuclear cells from patients infected with HIV-1. AIDS 1999;13(16):2239-50.
7. Wang LH, Begley J, St Claire RL, 3rd, Harris J, Wakeford C, Rousseau FS. Pharmacokinetic and pharmacodynamic characteristics of emtricitabine support its once daily dosing for the treatment of HIV infection. AIDS Res Hum Retroviruses 2004;20(11):1173-82.
8. Rousseau FS, Wakeford C, Mommeja-Marin H, Sanne I, Moxham C, Harris J, et al. Prospective randomized trial of emtricitabine versus lamivudine short-term monotherapy in human immunodeficiency virus-infected patients. J Infect Dis 2003;188(11):1652-8.
9. Sax PE, Tierney C, Collier AC, Fischl MA, Mollan K, Peeples L, et al. Abacavir-lamivudine versus tenofovir-emtricitabine for initial HIV-1 therapy. N Engl J Med 2009;361(23):2230-40.
10. Daar E TC, Fischl M et al. ACTG 5202: Final Results of ABC/3TC or TDF/FTC with either EFV or ATV/r in Treatment -naive HIV-infected Patients. In: 17th Conference on Retroviruses & Opportunistic Infections (CROI 2010). San Francisco.; 2010.
11. Daar ES, Tierney C, Fischl MA, Sax PE, Mollan K, Budhathoki C, et al. Atazanavir plus ritonavir or efavirenz as part of a 3-drug regimen for initial treatment of HIV-1. Ann Intern Med 2011;154(7):445-56.
12. Post FA, Moyle GJ, Stellbrink HJ, Domingo P, Podzamczer D, Fisher M, et al. Randomized comparison of renal effects, efficacy, and safety with once-daily abacavir/lamivudine versus tenofovir/emtricitabine, administered with efavirenz, in antiretroviral-naive, HIV-1-infected adults: 48-week results from the ASSERT study. J Acquir Immune Defic Syndr 2010;55(1):49-57.

13. Gallant JE, DeJesus E, Arribas JR, Pozniak AL, Gazzard B, Campo RE, et al. Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. N Engl J Med 2006;354(3):251-60.
14. Pozniak AL, Gallant JE, DeJesus E, Arribas JR, Gazzard B, Campo RE, et al. Tenofovir disoproxil fumarate, emtricitabine, and efavirenz versus fixed-dose zidovudine/lamivudine and efavirenz in antiretroviral-naive patients: virologic, immunologic, and morphologic changes--a 96-week analysis. J Acquir Immune Defic Syndr 2006;43(5):535-40.
15. Arribas JR, Pozniak AL, Gallant JE, Dejesus E, Gazzard B, Campo RE, et al. Tenofovir disoproxil fumarate, emtricitabine, and efavirenz compared with zidovudine/lamivudine and efavirenz in treatment-naive patients: 144-week analysis. J Acquir Immune Defic Syndr 2008;47(1):74-8.
16. Campbell TB, Smeaton LM, Kumarasamy N, Flanigan T, Klingman KL, Firnhaber C, et al. Efficacy and safety of three antiretroviral regimens for initial treatment of HIV-1: a randomized clinical trial in diverse multinational settings. PLoS Med 2012;9(8):e1001290.
17. Svicher V, Alteri C, Artese A, Forbici F, Santoro MM, Schols D, et al. Different evolution of genotypic resistance profiles to emtricitabine versus lamivudine in tenofovir-containing regimens. J Acquir Immune Defic Syndr 2010;55(3):336-44.
18. Marcelin AG, Charpentier C, Wirden M, Landman R, Valantin MA, Simon A, et al. Resistance profiles of emtricitabine and lamivudine in tenofovir-containing regimens. J Antimicrob Chemother 2012;67(6):1475-8.
19. Maserati R, De Silvestri A, Uglietti A, Colao G, Di Biagio A, Bruzzone B, et al. Emerging mutations at virological failure of HAART combinations containing tenofovir and lamivudine or emtricitabine. AIDS 2010;24(7):1013-8.
20. Darin K SK, Meloni S, Rawizza H, Kanki P. Clinical and virological outcomes of six first line regimens in a large ART program in Nigeria. In: The 10th International AIDS Conference. Vienna: International AIDS Society.; 2010.
21. Tang MW, Kanki PJ, Shafer RW. A review of the virological efficacy of the 4 World Health Organization-recommended tenofovir-containing regimens for initial HIV therapy. Clin Infect Dis 2012;54(6):862-75.
22. Stanford HIV Database; algorithm on HIV mutations and drug susceptibility. Available at: http://hivdb.stanford.edu/. Accessed on: 30 August 2013. In.

23. Nijhuis M, Boucher CA, Schuurman R. Sensitive procedure for the amplification of HIV-1 RNA using a combined reverse-transcription and amplification reaction. Biotechniques 1995;19(2):178-80, 182.
24. Yuen GJ, Lou Y, Bumgarner NF, Bishop JP, Smith GA, Otto VR, et al. Equivalent steady-state pharmacokinetics of lamivudine in plasma and lamivudine triphosphate within cells following administration of lamivudine at 300 milligrams once daily and 150 milligrams twice daily. Antimicrob Agents Chemother 2004;48(1):176-82.
25. Rey D, Hoen B, Chavanet P, Schmitt MP, Hoizey G, Meyer P, et al. High rate of early virological failure with the once-daily tenofovir/lamivudine/nevirapine combination in naive HIV-1-infected patients. J Antimicrob Chemother 2009;63(2):380-8.
26. Towner W KH, LaRieve M et al. Efficacy of a once daily (QD) regimen of nevirapine (NVP), lamivudine (3TC) and tenofovir (TDF) in treatment-naive HIV infected patients: a pilot study. In: Abstracts of the Seventh International Congress on Drug Therapy in HIV infection, Glasgow, 2004. Abstract P49.
27. When To Start C, Sterne JA, May M, Costagliola D, de Wolf F, Phillips AN, et al. Timing of initiation of antiretroviral therapy in AIDS-free HIV-1-infected patients: a collaborative analysis of 18 HIV cohort studies. Lancet 2009;373(9672):1352-63.
28. Doyle T, Smith C, Vitiello P, Cambiano V, Johnson M, Owen A, et al. Plasma HIV-1 RNA detection below 50 copies/ml and risk of virologic rebound in patients receiving highly active antiretroviral therapy. Clin Infect Dis 2012;54(5):724-32.
29. Charpentier C, Landman R, Laouenan C, Joly V, Hamet G, Damond F, et al. Persistent low-level HIV-1 RNA between 20 and 50 copies/mL in antiretroviral-treated patients: associated factors and virological outcome. J Antimicrob Chemother 2012;67(9):2231-5.
30. Lohse N, Kronborg G, Gerstoft J, Larsen CS, Pedersen G, Pedersen C, et al. Virological control during the first 6-18 months after initiating highly active antiretroviral therapy as a predictor for outcome in HIV-infected patients: a Danish, population-based, 6-year follow-up study. Clin Infect Dis 2006;42(1):136-44.

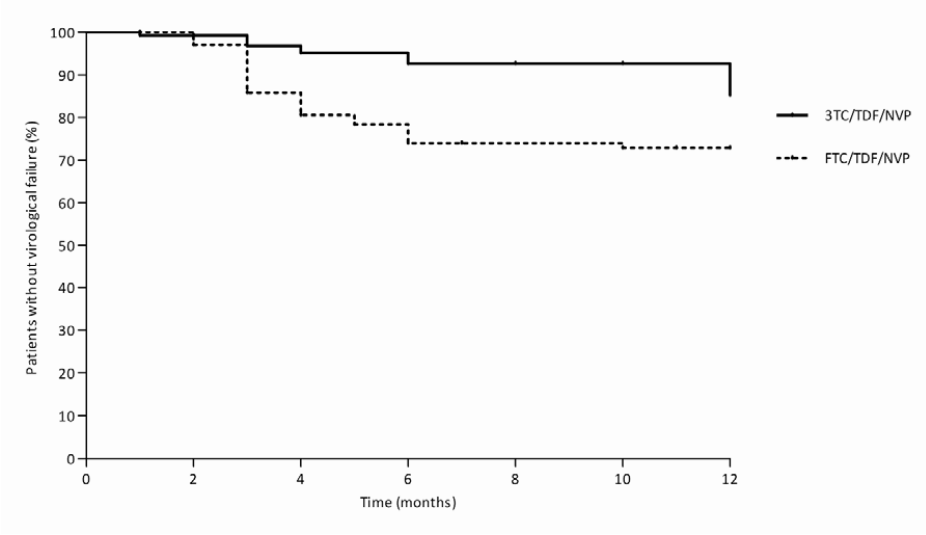

Figure 1 Kaplan Meier curves depicting the time to virological failure within the first year of treatment of therapy-naive HIV-1 patients that initiated lamivudine/tenofovir/nevirapine (dotted line) or emtricitabine/tenofovir/nevirapine. The time to virological failure was significantly shorter for HIV-1 patients that initiated lamivudine (Log-Rank: P=0.009). The Cox proportional hazards analysis confirmed an increased hazard ratio of virological failure for lamivudine compared to emtricitabine (2.01, interquartile range: 1.09 - 3.70, P= 0.026), adjusted for a baseline CD4 count <200 cells/mm^3 (3.28, 1.68 - 6.41) and HIV-RNA ≥100,000 copies/ml (2.22, 1.19 - 4.15).

Table 1 Baseline characteristics of therapy-naive HIV-1 infected patients without baseline resistance that initiated treatment with either lamivudine/tenofovir/nevirapine or emtricitabine/tenofovir/nevirapine.

	3TC/TDF/NVP (n=86)		FTC/TDF/NVP (n=110)		*P-value*
Male Sex, N (%)	67	(77.9)	85	(77.3)	0.920
Age years, mean (SD)	41	(10)	41	(11)	0.920
Starting year, median (IQR)	2004	(2003-2005)	2009	(2008-2010)	0.001
Transmission, N (%)					
MSM	41	(47.7)	66	(60.0)	
Heterosexual	31	(36.0)	36	(32.7)	
Intravenous drug abuse	5	(5.8)	2	(1.8)	0.243
Unknown	7	(8.1)	4	(3.6)	
Other	2	(2.3)	2	(1.8)	
Origin, N (%)					
Resource rich countries	51	(59.3)	71	(64.5)	
African countries	29	(33.7)	30	(27.3)	0.620
Other countries	6	(7.0)	9	(8.2)	
Median HIV-RNA (copies/ml)	94,900		57,050		0.014
<500, N (%)	1	(1.2)	0	(0.0)	
500-99,999, N (%)	44	(51.2)	71	(64.5)	0.104
≥100,000, N (%)	41	(47.7)	39	(35.5)	
Median CD4 cell count, cells/mm^3 [IQR]	200	(90-250)	225	(140-290)	0.020
<200, N (%)	42	(48.8)	45	(40.9)	
200–349, N (%)	42	(48.8)	55	(50.0)	0.116
≥350, N (%)	2	(2.3)	10	(9.1)	

All categorical variables are shown as number (%).
Categorical data is compared by using the χ^2 test and continuous data by the independent T test or Mann-Whitney U test if appropriate.
3TC: lamivudine, FTC: emtricitabine, IQR: interquartile range, MSM: men having sex with men, NVP: nevirapine, SD: standard deviation, TDF: tenofovir.

Table 2 Univariate comparison of virological responses within the first year of treatment between therapy-naive HIV-1 infected patients that initiated either lamivudine/tenofovir/nevirapine or emtricitabine/tenofovir/nevirapine.

	Virological succes (n=153)		**Virological failure (n=43)**		***P-value***
3TC/TDF/NVP (n=86)	60	(69.8%)	26	(30.2%)	
FTC/TDF/NVP (n=110)	93	(84.5%)	17	(15.5%)	0.010
Baseline HIV-RNA, copies/mL					
3TC/TDF/NVP					
median HIV-RNA	88,100		≥100,000		0.100
0 - 99,999	36	(80.0%)	9	(20.0%)	
≥100,000	24	(58.5%)	17	(41.5%)	0.030
FTC/TDF/NVP					
median HIV-RNA	49,000		≥100,000		0.035
0 - 99,999	64	(90.1%)	7	(9.9%)	
≥100,000	29	(74.4%)	10	(25.6%)	0.028
Baseline CD4 count, cells/mm^3					
3TC/TDF/NVP					
median CD4 count (IQR)	210	(130-250)	85	(40-210)	0.059
<200	24	(57.1%)	18	(42.9%)	
≥200	36	(81.8%)	8	(18.2%)	0.013
FTC/TDF/NVP					
median CD4 count (IQR)	250	(170-310)	60	(20-180)	0.002
<200	32	(71.1%)	13	(28.9%)	
≥200	61	(93.8%)	4	(6.2%)	0.001

All categorical data are shown as number (%).
Categorical data is compared by using the χ^2 test and continuous data by the Mann-Whitney U test.
3TC: lamivudine, FTC: emtricitabine, NVP: nevirapine, TDF: tenofovir.

Table 3 Multivariate logistic regression model on virological failure for lamivudine/tenofovir/nevirapine compared to emtricitabine/tenofovir/nevirapine containing antiretroviral regimens, adjusted for baseline CD4 cell count and HIV-RNA.

	Odds Ratio	**(95% Confidence Interval)**	***P- value***
3TC/TDF/NVP regimen	2.19	(1.05 - 4.59)	0.037
Baseline HIV-RNA ≥100,000 copies/mL	2.62	(1.25 - 5.48)	0.011
Baseline CD4 count <200 cells/mm^3	4.00	(1.86 - 8.62)	<0.001

Table 4 Genotypic testing results of the acquired resistance to reverse transcriptase in therapy-naive HIV-1 patients without baseline resistance with virological failure on lamivudine/tenofovir/nevirapine or emtricitabine/tenofovir/nevirapine containing antiretroviral regimens.

	3TC/TDF/NVP[1] (n=25)		**FTC/TDF/NVP[1] (n=16)**	
NRTI RAM, N (%)				
K65R	7	(28.0)	5	(31.2)
D67N	1	(4.0)	0	
T69A/D/N	3	(12.0)	1	(6.2)
Y115F/R	1	(4.0)	1	(6.2)
M184I/V	17	(68.0)	9	(56.2)
Non-NRTI RAM, N (%)				
K101E/Q	0		3	(18.8)
K103N	3	(12.0)	2	(12.5)
V106A	3	(12.0)	0	
Y181C/I	15	(60.0)	9	(56.2)
Y188C	1	(4.0)	0	
G190A	3	(12.0)	0	
Genotypic resistance testing, N (%)				
No mutations	2	(8.0)	1	(6.2)
HIV-RNA <1000 copies/mL	0		5	(31.2)
≥ NRTI mutation	1	(4.0)	0	
≥ Non-NRTI mutation	2	(8.0)	0	
≥ NRTI and Non-NRTI mutation	20	(80.0)	10	(62.5)
≥ NRTI and/or Non-NRTI mutation	23	(92.0)	10	(62.5)

[1] 1 sample was unavailable in each treatment group for genotypic testing.
All variables are shown as number (%).
3TC: lamivudine, FTC: emtricitabine, NVP: nevirapine, (non-)NRTI: (non-)nucleoside reverse transcriptase inhibitor, RAM: resistance associated mutation, TDF: tenofovir.

Chapter 5

Hepatitis B virus prevalence, risk factors and genotype distribution in HIV infected patients from West Java, Indonesia

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Published in *Journal of Clinical Virology, 2014; 59(4):235-41*

Abstract

Background Indonesia currently faces both an increasing HIV incidence and a high hepatitis B virus (HBV) burden.

Objective The objective of our study is to examine the prevalence, risk factors, and genotypic distribution of HBV infection among HIV infected patients in West Java, Indonesia.

Study design A cross sectional study was conducted among a cohort of HIV infected patients in 2008. Demographic and disease related variables were compared between HBV negative and positive patients. Logistic regression was applied to determine risk factors for HBV co-infection. HBV and HIV genotyping was performed in co-infected patients.

Results Of 636 HIV-infected patients, the rate of HBV co-infection was 7%. The proportion of males was higher in HBV/HIV co-infected patients than in HIV mono-infected patients (93% *vs.* 72%, P=0.001). A history of injecting drug use (IDU), but not tattooing, was associated with HBV co-infection [*P*=0.035 OR 2.41, (95% CI 1.06-5.47)]. In the HIV and HBV treatment naive patients, CD4 cells counts <50 cells/mm^3, HIV-RNA plasma ≥10,000 copies/ml and AST level above normal were more often found in patients with high HBV-DNA levels (≥20,000 IU/ml) as compared to those with low HBV DNA (<20.000 copies/ml)(*P*<0.05). As in the general population, B3 was the dominant subtype in HBV co-infected patients.

Conclusions Both the prevalence of active HBV infection as the genotype distribution among HIV infected individuals is similar to the overall population in Java. However, an increased prevalence was observed in men with a history of IDU, underlining the need for routine HBV screening and monitoring.

Background

The worldwide number of people living with HIV is currently approximately 35 million. Estimates suggest that approximately 2-4 million of these HIV-infected patients also have a chronic HBV (hepatitis B virus) infection. [1] Co-infection with HIV and HBV can accelerate the progression of liver diseases resulting in fibrosis and hepatocelullar carcinoma. [2, 3] On the other hand, the progression of HIV-associated diseases and immunological and virological responses to highly active antiretroviral therapy (HAART) do not generally appear be affected by the presence of HBV[4-8]; although some studies reported that in regions in which HBV is highly endemic and many HBV-infected patients have advanced immunosuppression due to HIV, HIV-HBV co-infected patients have a significantly lower HIV treatment success rate. [9]

In Indonesia, hepatitis B infection is endemic and the HIV epidemic expanded significantly in recent years. [10] Low social economic background was associated with HBV infection in this country[11], while HIV infection was majorly driven by injecting drug use in all provinces, except Papua. [12] However, the prevalence, risk factors, as well as genotypic distribution of HIV-HBV co-infection in Indonesia have not been investigated using a large study cohort. Because the impact of HIV-HBV co-infection varies among countries worldwide, understanding these epidemiological factors is particularly relevant. Therefore, in this study we investigated HBV co-infection in a large cohort of HIV infected patients from West Java, Indonesia where injecting drug use (IDU) has been a major route of HIV transmission.

Objectives

We examined the prevalence, risk factors, genotypic distribution of HBV infection among HIV-infected patients in West Java, Indonesia.

Study Design

Setting and study population

This study was part of the European Commission funded IMPACT program aiming to prevent, control, and treat HIV among IDU in West-Java, Indonesia.[13] A cross-sectional epidemiological

study of hepatitis B virus was conducted in 2008, recruited adult HIV-infected patients who visited the HIV clinic of Hasan Sadikin Hospital (N=590), the main tertiary referral hospital of West Java, the methadone clinic of Bandung (N=37) and the clinic of Banceuy prison (N=9), the main narcotic prison of West Java. All medical check-ups and treatments were provided free of charge and HAART (Highly Active Antiretroviral Therapy) was provided according to Indonesian guidelines.[12] The presence of HBV surface antigen (HBsAg) was assessed from stored frozen plasma from all recruited patients (N=636), while HBV-DNA was assessed from those patients where sufficient plasma samples were available for testing (N=584). Co-infection with HBV was defined as presence of HBsAg and/or HBV-DNA. From all participants a signed informed consent was obtained. The study was approved by Human Research Ethics Committee of the University of Padjadjaran, West-Java, Indonesia.

Demographic, clinical and laboratory data

We collected the following three sets of data: (i) patient demographics and baseline characteristics, including age, gender, and risk behavior related to HIV infection; (ii) status of their HIV infection, including CD4 cell count, plasma HIV-RNA level and (iii) HIV treatment history and, and liver function profile.

To define the HBV status two HBsAg serological assays which differ in their target recognition were used (Elecsys HBsAg II assay (Roche, Meylan, France) and LIAISON® XL MUREX HBSAG QUANT XLassay (Diasorin, Saluggia, Italy). In-house assays were used for all HBV [14] and HIV nucleic acid detection. [15] CD4 cells counts were measured by Facscount flow cytometry technology (BD Biosciences, Jakarta, Indonesia). Alanine amino transaminase (ALT) and Aspartate amino transaminase (AST) levels were used to assess liver function of all patients

Molecular assays

For HBV genotyping, the Pre-S1, Pre-S2, S, X and part of pre-core genes (nt 2822-1822) were amplified using two overlapping primers sets (S1 (5'-gtatgttgcccgtttgtcctc-3', nt 461-481) and Pr109 (5'-aaaagttgcatggtgctgg-3', nt 1808-1822), Pr1 (5'-gggtcaccatattcttggg-3', nt 2822-2840) and YMDD-2 (5'-acccatctttttgttttgttagg-3', nt 840-863)). For HIV genotyping, the reverse

transcriptase and protease genes were amplified using in-house primers. [16] The amplified products were analyzed by the Sanger sequencing method and aligned by CLUSTALW program. A phylogenetic tree was constructed by neighbor-joining method and genetic distance was calculated by the Kimura distance formula (MEGA5 software, www.megasoftware.net).

Statistical analysis

Data were analyzed with SPSS version 20 (IBM). Statistical significance was taken as $P<0.05$. Characteristics were compared using the Chi-square test for categorical data and the Mann-Whitney test for continuous data. Factors associated with HBV co-infection were analyzed by logistic regression models. Multivariate models included all variables that were significant at the $P<0.10$ level in univariate analysis.

Results

Prevalence of HBsAg and HBV-DNA

In total, 636 HIV patients were recruited in this study. In 594 patients, the presence of HBsAg was analyzed using both assays (Elecsys and LIAISON® XL assays), while in 42 patients only a single assay (Elecsys) could be used due to insufficient amounts of plasma. Of 594 patients tested with two assays, 38 patients (6.4%) were HBsAg positive (28 positive at both assays, 4 positive only in Elecsys assay, and 6 positive only in Diasorin assay). From the 42 individuals tested with the Elecsys, seven were HBsAg positive (17%).

HBV-DNA measurements were conducted in 584 available samples. Out of these samples, 24 (4.1%) were positive with median of 6.1 $\log_{10}$ IU/ml (Interquartile range (IQR), 3.6-9.4 $\log_{10}$ IU/ml). Twenty-three of them had both HBsAg and HBV-DNA detected. Occult HBV infection, defined as presence of HBV-DNA with undetectable HBsAg, was observed in one patient only (0.2%) with a very high HBV-DNA load, 9.4 $\log_{10}$ IU/ml.

Overall, HBV co-infection was found in 46 patients (7%) while the prevalence in HAART treated patients (17/217 (8%)) did not significantly differ from the prevalence in treatment naïve patients (29/419 (7%)).

Characteristics of patients with and without HBV co-infection

Baseline demographic and clinical characteristics were compared by HBV infection status (Table 1). In general, most of the HBV positive patients were young men (73%) with a history of injecting drug use (IDU) (63%) and presented with advanced HIV disease characterized by a low CD4 cell counts (median 114 cells/mm^3,IQR 44-269). In HIV-HBV co-infected patients, male gender, IDU, and tattooing were more often found than in the HIV mono-infected patients (P<0.05). There was no statistical difference between two groups with respect to WHO HIV disease status. On the other hand, ALT levels were higher in HBV/HIV co-infected patients relatively to HIV mono-infected patients (Median 46 IU/L in HBV/HIV co-infected patients *vs.* 33 IU/L in HIV mono-infected patients, P=0.016).

Factors associated with HBV co-infection

To identify risk factors for HBV co-infection, socio-demographic characteristics of all recruited patients were studied in a logistic regression model. Male gender was excluded in the statistical analysis, because of its strong association with both HBV and HIV infection. Including male gender would therefore violate the absence multicollinearity assumption that is a key in logistic regression analysis. In multivariate analysis, a history of IDU was significantly associated with HBV co-infection [P=0.035 OR 2.41, (95% CI 1.06-5.47)] (Table 2).

To eliminate bias due to therapy the analysis of variables that relate to HIV and liver disease status was limited to HAART naïve patients (N=419). In an univariate model, none of the following variables: WHO disease stage 3 or 4, CD4 cells count <50 cells/mm^3, plasma HIV-RNA ≥10,000 copies/ml, AST and ALT above normal were associated with HBV co-infection (Table 3).

Characteristics of patients with high HBV-DNA level

To identify active HBV replication among HBsAg positive patients, which is defined as the HBV-DNA ≥20,000 IU/ml, we characterized HIV/HBV treatment naïve patients based on their HBV-DNA outcome. Of 419 HIV treatment naïve patients, 29 were both HBsAg and HBV-DNA positive. Among of these 29 patients, 15 had HBV-DNA <20,000 ml and 14 had HBV-DNA ≥20,000 IU/ml. Compared with patients with HBV-DNA <20,000 IU/ml, active HBV patients with HBV-DNA

≥20,000 IU/ml had lower CD4 cells count and higher HIV-RNA plasma level. Additionally, active HBV patients showed a higher proportion of AST level above normal (Table 4).

Distribution of HBV and HIV genotypes among co-infected patients

HBV sequences were available for 18 of 45 individuals (17 HBsAg positive and one HBsAg negative); in the remaining samples plasma HBV DNA levels were too low to generate a result (six patient less than 3 $\log_{10}$ IU/ml and 21 individuals less than 2 $\log_{10}$ IU/ml). HBV phylogenetic analysis included eighteen sequences from Indonesian HBV mono-infected patients, which were available in the GenBank. Similar genotype distribution was found between HBV mono- and co-infected patients. In co-infected patients, the most prevalent sub genotype was B3 (12/18), followed by sub genotypes D1 (3/18), B2 (2/18) and C1 (1/18). Sub genotype B3 was found in patients with and without a history of IDU. Interestingly, sub-genotypes B2, and D1 were only found in patients with a history of IDU. The occult HBV sample grouped with Indonesian HBV sub genotype D1 (Figure 1A).

Among 18 patients whom had available HBV sequences, 14 patients also had correspondent HIV sequences, while four patients had undetectable HIV-RNA. A phylogenetic analysis was conducted in 14 HIV samples, included sequences from HIV mono-infected individuals from this study. All patient samples were defined as HIV CRF01_AE. There were no clear separations between HIV mono- and co-infected sequences, neither between IDU and non-IDU sequences. The sample from occult HBV infected patient could not be analyzed due to a low plasma HIV-RNA level (Figure 1B).

Discussion

In this paper we investigated the prevalence of co-infection, risk factors and genotypic distribution of HBV in HIV infected patients in West Java, Indonesia.

The prevalence of HBV/HIV co-infection was 7%, which does not differ from HBV mono-infected prevalence in the general population of Java, Indonesia (5%). [17] However, this co-infection prevalence was lower compared with a previous study in East Java, which observed a co-infection prevalence of 15%. The socio-demographic background and the prevalence of HBV

in the general population were, however, similar between our study and the study in East Java. [18] Different interpretations of these differing results could be made. First, our lower observed prevalence might be due to the differences in the assays used. However, assessment of modern HBsAg assays showed comparable sensitivity among all commonly used methods. [19] In addition, we did not observe any significant differences in results by assay in our study (co-infection prevalence of 5.2% at Elecsys HBsAg assay *vs* 5.6% at LIAISON® XL MUREX HBSAG QUANT XL assay). Second, it may be that differences exist between the two groups studied. It is known that HBV co-infection rates increase in individuals with advanced stages of HIV disease. [20] Fewer patients with advanced HIV infection were recruited in our study compared with the study in East Java. This is supported by lower proportions of patients under HIV treatment (34%*vs* 98%), and of patients with CD4 cells counts below 200 cells/mm^3 (47% *vs. 72*%).The reason our study recruited patients earlier in the course of HIV infection is likely due to our ongoing comprehensive prevention and treatment program in West Java. [13]

In this study, we observed a higher probability of HBV co-infection in HIV patients with a history of IDU than in HIV patients without any history of IDU. A previous study from West Java, screening all incoming prisoners in the same narcotic prison from which we recruited part of our study subjects, found tattooing, but not IDU, to be significantly associated with detectable HBsAg [*P*=0.035 OR 2.41 (95% CI: 1.06–5.47)]. [21] However, we could not confirm the association between tattooing and HBV co-infection in our population, which mainly included patients from other sites. Compared to the number of patients that we recruited from HIV clinic of Hasan Sadikin hospital (590/636**),** fewer patients were recruited from the narcotic prison (9/636). Therefore, it appears that tattooing is a risk factor for HBV infection among prisoners but not for HBV infection among HIV infected patients in the hospital. On the other hand, we can not rule out that the association between a history of IDU and HBV co-infection that we found in this study was due to a bias in sample selection, given that most of our patients were having a history of IDU. Therefore, in order to confirm the transmission route of HBV infection among HIV infected individuals in Indonesia, further works involving more HIV risk groups need to be performed.

Our study showed comparable genotypic distribution of HBV in both HBV mono-infected and HIV/HBV co-infected patients. This similar HBV genotypic distribution was in

line with other studies from Belgian[22, 23] and Africa[24, 25] which found genotype A in general and HIV co-infected individuals. Thus, it was suggested that there are no preference of HBV genotypes with respect to HIV co-infection.

According to the WHO guideline [26], lamivudine or emtiricitabine and tenofovir therapy should be given to the HBV/HIV co-infected patients with active HBV replication, which in well-resourced settings is based on histological parameters obtained by liver biopsy and/or on HBV DNA testing. Those two diagnostic tools for identifying chronic active HBV patients are not available in developing settings. Given that the availability of tenofovir is limited in developing countries, it is urgently need to find alternative ways for identifying active HBV infection. In this study, we observed that low CD4 cells (<50 cells/mm^3), high HIV-RNA levels (≥10,000 copies/ml) and increased AST levels were often found in active HBV patients who previously did not received any HIV and/or HBV treatment. Therefore, measuring AST levels might be useful to screen for active HBV replication among HBV/HIV co-infected individuals where standard liver biopsy and/or HBV-DNA testing could not be performed. Nevertheless, confirmation studies with a longitudinal data set and a larger number of HBsAg positive samples need to be done.

This study may have clinical consequences for HBV/HIV treatment and prevention in Indonesia. First, the moderate rate of HBV co-infection which was found in this study requires adequate diagnostic tools for indentifying HBV/HIV co-infected patients who need treatment for their HBV infection. Second, tenofovir should be included in the first line ART regimen for HBV/HIV co-infected patients [26]. Third, in order to lower the risk of HBV/HIV co-infection which is more complex to treat than HIV mono-infection, HBV vaccination among HIV infected patients should be conducted, especially to those patients with male gender, a history of IDU and tattoo.

This study had several limitations. First, due to the cross-sectional nature, the diagnosis of HBV co-infection was based on the presence of HBsAg and HBV-DNA in patient plasma. For this reason, the natural history of hepatitis B phases in these co-infected patients could not be traced. Second, the samples size of our treatment-naïve group likely made it difficult to find more disease-related risk factors. Lastly, risk behavior related to HIV infection other than IDU and tattooing was not assessed. Despite of these limitations, our research provided a comprehensive overview of the epidemiology of HBV in HIV-infected patients in Indonesia.

In summary, a comparable prevalence and genotypic distribution of HBV were found in both HIV-infected patients and in general population. A history of IDU might play a role in HBV transmission among HIV infected individuals. Further research need to be done to investigate the impact of HBV co-infection on response to antiviral therapy and HIV disease progression in Indonesian cohort.

Funding

European Commission (Sixth Framework Programme; Specific Targeted Research Project no: 318／10150A – EC SANTE⁄2005⁄105-033) and Dept. of Virology, Erasmus Medical Center, the Netherlands.

Competing interests

None of the authors declares any conflict of interest.

Ethical approval

Ethical Committee of Hasan Sadikin Hospital/Medical Faculty of Universitas Padjadjaran, Bandung, Indonesia (No. 85/FKUP-RSHS/KEPK/Kep/EC/2006)

Acknowledgement

We thank to the staff and patients of the Hasan Sadikin Hospital, West Java, Indonesia, for their participation and the Banceuy Narcotic Prison for hosting the cohort study of blood borne infection in their institution. We are grateful to Dept. of Virology, Erasmus Medical Center, and the Netherlands for donating reagents for sample testing and for additional training support in West Java. We acknowledge the financial support of the IMPACT program that has been provided by the European Commission (Sixth Framework Programme; Specific Targeted Research Project no: 318／10150A – EC SANTE⁄2005⁄105-033).

References

1. Alter MJ. Epidemiology of viral hepatitis and HIV co-infection. J Hepatol 2006;44(1 Suppl):S6-9.

2. Li YJ, Wang HL, Li TS. Hepatitis B virus/human immunodeficiency virus coinfection: interaction among human immunodeficiency virus infection, chronic hepatitis B virus infection, and host immunity. Chin Med J (Engl) 2012;125(13):2371-7.
3. Thio CL. Hepatitis B and human immunodeficiency virus coinfection. Hepatology 2009;49(5 Suppl):S138-45.
4. Wang H, Li Y, Zhang C, Han Y, Zhang X, Zhu T, et al. Immunological and virological responses to cART in HIV/HBV co-infected patients from a multicenter cohort. AIDS 2012.
5. Chadwick D, Ankcorn M, Sarfo F, Phillips R, Fox Z, Garcia A, et al. Outcomes of starting first-line antiretroviral therapy in hepatitis B virus/HIV-coinfected patients in Ghana. J Antimicrob Chemother 2012.
6. Hoffmann CJ, Seaberg EC, Young S, Witt MD, D'Acunto K, Phair J, et al. Hepatitis B and long-term HIV outcomes in coinfected HAART recipients. AIDS 2009;23(14):1881-9.
7. Idoko J, Meloni S, Muazu M, Nimzing L, Badung B, Hawkins C, et al. Impact of hepatitis B virus infection on human immunodeficiency virus response to antiretroviral therapy in Nigeria. Clin Infect Dis 2009;49(8):1268-73.
8. Anggorowati N, Yano Y, Heriyanto DS, Rinonce HT, Utsumi T, Mulya DP, et al. Clinical and virological characteristics of hepatitis B or C virus co-infection with HIV in Indonesian patients. J Med Virol 2012;84(6):857-65.
9. Sheng WH, Chen MY, Hsieh SM, Hsiao CF, Wang JT, Hung CC, et al. Impact of chronic hepatitis B virus (HBV) infection on outcomes of patients infected with HIV in an area where HBV infection is hyperendemic. Clin Infect Dis 2004;38(10):1471-7.
10. UNAIDS. UNAIDS global report 2010. 2010.
11. Akbar N, Basuki B, Mulyanto, Garabrant DH, Sulaiman A, Noer HM. Ethnicity, socioeconomic status, transfusions and risk of hepatitis B and hepatitis C infection. J Gastroenterol Hepatol 1997;12(11):752-7.
12. Wisaksana R, Indrati AK, Fibriani A, Rogayah E, Sudjana P, Djajakusumah TS, et al. Response to first-line antiretroviral treatment among human immunodeficiency virus-infected patients with and without a history of injecting drug use in Indonesia. Addiction 2010;105(6):1055-61.
13. Alisjahbana B, Susanto H, Roesli R, Yusuf H, Hinduan Z, Mase JC, et al. Prevention, control and treatment of HIV-AIDS among injecting drug use in Bandung, Indonesia. Acta Med Indones 2009;41 Suppl 1:65-9.
14. Pas SD, Fries E, De Man RA, Osterhaus AD, Niesters HG. Development of a quantitative real-time detection assay for hepatitis B virus DNA and comparison with two commercial assays. J Clin Microbiol 2000;38(8):2897-901.

15. Fibriani A, Farah N, Kusumadewi I, Pas SD, van Crevel R, van der Ven A, et al. Low cost HIV-1 quantitative RT-PCR assay in resource-limited settings: improvement and implementation. J Virol Methods 2012;185(1):118-23.
16. Nijhuis M, Boucher CA, Schuurman R. Sensitive procedure for the amplification of HIV-1 RNA using a combined reverse-transcription and amplification reaction. Biotechniques 1995;19(2):178-80, 182.
17. Mulyanto, Depamede SN, Surayah K, Tsuda F, Ichiyama K, Takahashi M, et al. A nationwide molecular epidemiological study on hepatitis B virus in Indonesia: identification of two novel subgenotypes, B8 and C7. Arch Virol 2009;154(7):1047-59.
18. Utsumi T, Yano Y, Lusida MI, Nasronudin, Amin M, Juniastuti, et al. Detection of highly prevalent hepatitis B virus co-infection with HIV in Indonesia. Hepatol Res 2012.
19. Geretti AM, Patel M, Sarfo FS, Chadwick D, Verheyen J, Fraune M, et al. Detection of highly prevalent hepatitis B virus coinfection among HIV-seropositive persons in Ghana. J Clin Microbiol 2010;48(9):3223-30.
20. Attia KA, Eholie S, Messou E, Danel C, Polneau S, Chenal H, et al. Prevalence and virological profiles of hepatitis B infection in human immunodeficiency virus patients. World J Hepatol 2012;4(7):218-23.
21. Nelwan EJ, Van Crevel R, Alisjahbana B, Indrati AK, Dwiyana RF, Nuralam N, et al. Human immunodeficiency virus, hepatitis B and hepatitis C in an Indonesian prison: prevalence, risk factors and implications of HIV screening. Trop Med Int Health 2010;15(12):1491-8.
22. Micalessi MI, De Cock L, Vranckx R. Hepatitis B virus (HBV) genotyping in Belgian patients with chronic HBV infection. Clin Microbiol Infect 2005;11(6):499-501.
23. Pourkarim MR, Lemey P, Amini-Bavil-Olyaee S, Houspie L, Verbeeck J, Rahman M, et al. Molecular characterization of hepatitis B virus strains circulating in Belgian patients co-infected with HIV and HBV: overt and occult infection. J Med Virol 2011;83(11):1876-84.
24. Kramvis A, Kew MC. Epidemiology of hepatitis B virus in Africa, its genotypes and clinical associations of genotypes. Hepatol Res 2007;37(s1):S9-S19.
25. Makondo E, Bell TG, Kramvis A. Genotyping and molecular characterization of hepatitis B virus from human immunodeficiency virus-infected individuals in southern Africa. PLoS One 2012;7(9):e46345.
26. WHO. Antiretroviral therapy for HIV infection in adults and adolescents Recommendations for a public health approach 2010 revision. 2010.

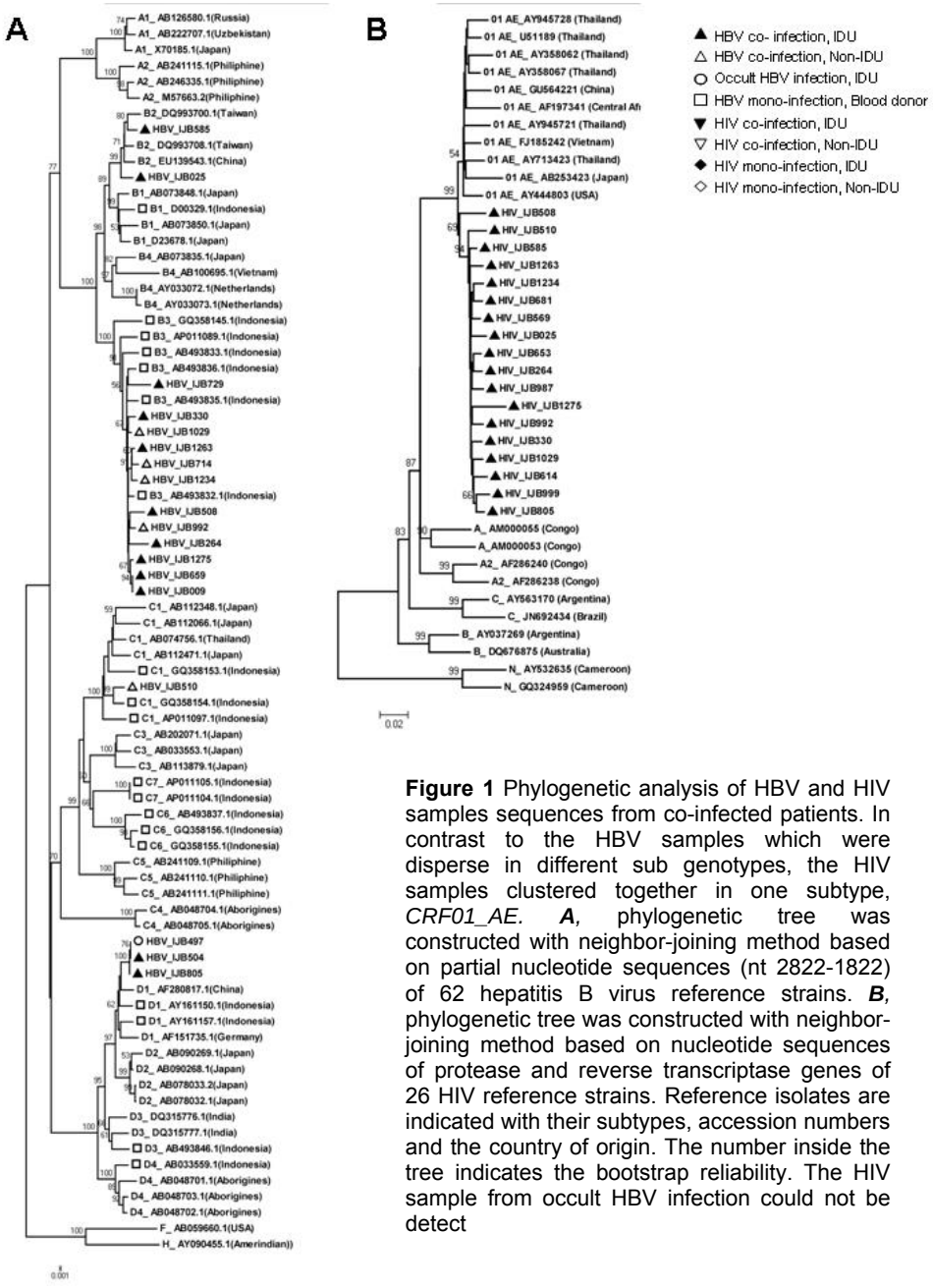

Figure 1 Phylogenetic analysis of HBV and HIV samples sequences from co-infected patients. In contrast to the HBV samples which were disperse in different sub genotypes, the HIV samples clustered together in one subtype, *CRF01_AE.* ***A***, phylogenetic tree was constructed with neighbor-joining method based on partial nucleotide sequences (nt 2822-1822) of 62 hepatitis B virus reference strains. ***B***, phylogenetic tree was constructed with neighbor-joining method based on nucleotide sequences of protease and reverse transcriptase genes of 26 HIV reference strains. Reference isolates are indicated with their subtypes, accession numbers and the country of origin. The number inside the tree indicates the bootstrap reliability. The HIV sample from occult HBV infection could not be detect

Table 1 Characteristics of patients based on HBV co-infection status

Characteristic	Total patients (N = 636)	HIV mono-infection (N = 590)	HBV/HIV co-infection (N = 46)	*P-value*[a]
Baseline and socio-demographics				
Gender, *N* (%)				
Male gender	465 (73)	422 (72)	43 (93)	**0.001**
Female	171 (27)	168 (28)	3 (7)	
History of IDU, *N* (%)				
Yes	403 (63)	365 (62)	38 (83)	
No	185 (29)	180 (30)	5 (10)	**0.013**
Unknwon	48 (8)	45 (8)	3 (7)	
Tattoo, *N* (%)				
Yes	207 (33)	184 (31)	23 (50)	
No	351 (55)	335 (57)	16 (35)	**0.013**
Unknown	78 (12)	71 (12)	7 (15)	
HIV disease status				
WHO, *N* (%)				
Stage 1 or 2	127 (20)	120 (20)	7 (15)	
Stage 3 or 4	326 (51)	302 (52)	24 (52)	0.663
Unknown	183 (29)	168 (28)	15 (33)	
Median CD4 count, cells/mm^3 (IQR)	114 (44-269)	214 (52-351)	114 (44-269)	0.168
HIV-RNA detected, *N* (%)				
Negative	225 (36)	208 (35)	17 (37)	
Positive	357 (56)	330 (56)	27 (59)	0.596
Not tested	54 (8)	52 (9)	2 (4)	
Median HIV-RNA, $\log_{10}$ copies/ml (IQR)	4,9 (4.3-5.4)	4.9 (4.3-5.4)	5.1 (4.7-5.6)	0.087
Liver disease status				
Median ALT, IU/L (IQR)	33 (19-58)	33 (18-55)	46 (26-69)	**0.016**
Median AST, IU/L (IQR)	32 (23-52)	32 (23-52)	40 (27-51)	0.177
Treatment history				
Naïve, *N* (%)	419 (66)	390 (66)	29 (63)	0.673
Treated, *N* (%)	217 (34)	200 (34)	17 (37)	
NRTI backbone of ARV therapy[b],*N* (%)				
Zidovudine + lamivudine	158 (73)	147 (73)	11 (65)	
Stavudine + lamivudine	58 (26)	52 (26)	6 (35)	0.552
Tenovofir + lamivudine	1 (1)	1 (1)	0 (0)	
NNRTI / PI component of ARV therapy[b], *N* (%)				
Nevirapine	140 (64)	130 (65)	10 (59)	
Efavirenz	74 (34)	67 (33)	7 (41)	0.640
Lopinavir + ritonavir	3 (2)	3 (2)	0 (0)	
Median treatment duration, months[b] (IQR)	22 (12.5-32)	23 (13-32)	14 (9-26)	0.057
HBV drugs resistance mutations[b], *N* (%)	1 (1)	N.A	1 (6)	N.A.

[a]Characteristics were compared between HIV mono-infected and HBV/HIV co-infected patients using the Chi-square test for catergorical variables and the Mann-Whitney test for countinuous variables.
[b]Applied for treated patients
Abbrevations: IQR, interquartile range; IDU, injecting drug use; WHO, world health organization; ALT, alanine transferase; AST, aspartate transferase; ART, antiretroviral therapy; NRTI, nucleoside reverse trasncriptase inhobitor, NNRTI, non-nucleoside reverse trancriptase; N.A. not applicable.

Table 2 Baseline socio-demographic factors associated with HBV co-infection in 636 HIV infected patients

	Total patients	HBV co-infection	Univariate		Multivariate[a]	
	(N)	(N)	OR (95% CI)	*P-value*	OR (95% CI)	*P-value*
Age ≥30 years	148	9	0.75 (0.34-1.54)	0.451	N.A.	N.A.
IDU (Yes)	403	38	2.92 (1.34-6.39)	0.007	2.41 (1.06-5.47)	**0.035**
Tattoo (Yes)	207	23	2.21 (1.21-4.04)	0.010	1.70 (0.90-3.21)	0.101

[a]Multivariate models included all variables that were significant at the *P*<0.10 level in univariate analysis.
N.A. stands for not applicable

Table 3 Diseases related factors associated with HBV co-infection in 419 HIV treatment naïve patients

	Total naïve patients (N)	HBV/HIV co-infection (N)	Univariate		Multivariate[a]	
			OR (95% CI)	*P-value*	OR (95% CI)	*P-value*
HIV disease status						
WHO Stage 3 or 4	164	13	1.21 (0.52-2.39)	0.779	N.A.	N.A.
CD4 cells count <50 cells/mm^3	129	11	1.23 (0.56-2.69)	0.593	N.A.	N.A.
HIV-RNA plasma ≥10,000 copies/ml	262	23	1.87 (0.74-4.71)	0.183	N.A.	N.A.
Liver disease status						
ALT Above normal[b]	104	11	1.68 (0.76-3.67)	0.194	N.A.	N.A.
AST Above normal[c]	135	12	1.33 (0.61-2.87)	0.463	N.A.	N.A.

[a]Multivariate models included all variables that were significant at the *P*<0.10 level in univariate analysis.
[b]ALT normal for female: 5-38 IU/L; male: 10-50 IU/L.
[c]AST normal for female: 6-34IU/L; male: 8-40 IU/L.
N.A. stands for not applicable

Table 4 Characteristics of 29 HIV treatment naïve-HbsAg positive patients based on HBV-DNA out come

Characteristics	No. of patients with HBV-DNA <20,000 IU/ml N(%)	No. of patients with HBV-DNA ≥20,000 IU/ml N (%)	*P-value* [a]
Total	15	14	
Baseline and socio-demographics			
Male	13 (87)	13 (92)	0.584
Age ≥31years	2 (13)	2 (14)	0.343
IDU	13 (87)	8 (62)	0.075
Tattoo	5 (33)	6 (46)	0.151
HIV disease stage			
WHO stage 3 or 4	6 (40)	7 (54)	0.315
CD4 cells count <50 cells/mm^3	2 (13)	9 (69)	**0.005**
HIV-RNA plasma ≥10,000 copies/ml	9 (60)	14 (100)	**0.008**
Liver disease stage			
ALT above normal[b]	5 (33)	6 (43)	0.597
AST above normal[c]	4 (27)	8 (62)	**0.037**

[a]Characteristics were compared between HBV-DNA <20,000 IU/ml and ≥20,000 IU/mL patients using the Chi-square test
[b]ALT normal for female: 5-38 IU/L; male: 10-50 IU/L.
[c]AST normal for female: 6-34IU/L; male: 8-40 IU/L.
N.A. stands for not applicable

Chapter 6

High incidence of Hepatitis C virus co-infection in HIV-1 positive injecting drug users from West Java, Indonesia

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Submitted

Abstract

Background We studied the risk factors and genotype distribution of HCV in an HIV-1 infected cohort in Bandung, Indonesia

Results From 619 individuals tested 67% (419) had HCV-antibodies: male gender, age below 30 and a history of IDU or tattooing was associated with HCV-Ab positivity. Of 322 HCV antibody positive patients, 265 (82%) were chronically infected. The most prevalent HCV sub genotype was 1a (50%), followed by 3k>1c>4a>3a>1b. Sub genotypes 4a and 1a, were recently introduced.

Conclusions A high prevalence of chronic HCV co-infection due to several recent transmission events was found in Bandung.

Introduction

Worldwide, approximately 4–5 million people are co-infected with HCV and HIV. [1] The prevalence of HCV co-infection depends on the HIV transmission route from 10% among those with high-risk sexual behavior to 90-100% for injecting drug users (IDU). [2]

HIV infection can alter the natural history of HCV infection in several ways. First, of all the HCV antibody response can be delayed, [3], secondly, the riks for development of chronic HCV infection increases] [4] Third, higher plasma HCV RNA levels are found, associated with accelerated progression to fibrosis and cirrhosis [5]

HCV prevalence in Indonesia has always been moderate (<3%) but the HIV epidemic fuelled by IDU is among the fastest growing in Asia [6] . In one study a high frequency of HCV co-infection has been reported form Indonesia, particularly among IDU. [7] In this study we investigated the prevalence and risk factors for chronic HCV co- infection in a large HIV cohort in West Java, Indonesia [8,9,10]

Methods

Setting and study population

This study was part of the Integrated Management for AIDS Prevention and Treatment Care (IMPACT) program.[9]. All HIV-positive patients above 14 years of age presenting in 2008 at Hasan Sadikin hospital, the referral hospital for HIV in West Java (population 40 million), were included in a cohort study. Data were collected for including age, sex, history of injecting drug use, body mass index (BMI, kg/m^2), stage of HIV infection and HIV treatment history, included antiviral regimens and treatment duration. HCV antibody was tested using the Elecsys Anti-HCV assay (Roche Diagnostics, Basel IA, Switzerland). HCV-RNA tests were performed in HCV-antibody (Ab) positive patients. Chronic HCV infection was defined by the presence of HCV-RNA in plasma. All participants provided written informed consent. This study was approved by the Human Research Ethics Committee of the University of Padjadjaran, West Java, Indonesia.

Molecular assays

Nucleic acids were extracted from 200-µl frozen stored plasma samples using MagnaPure LC (Roche Diagnostics, GmBH, Germany). In-house real-time HBV and HIV PCR assays were performed as described previously. [8] [11] HCV-RNA was detected by amplifying the extracts with the following primer set: 5-gcagaaagcgtctagccatggcgttag-3 and 5-caagcaccctatcaggcagtaccacaa-3; the amplified product was probed with the following two probes: 5-FAM-ccatagtggtctgcggaaccggtgagtac-BHQ1-3 and 5-FAM-accggaatcgccgggatgaccgggtcctttt-BHQ1-3. An HCV viral lysis stock was used as a standard for quantification.

For HCV genotyping, the RNA-dependent polymerase NS5b fragment and 5'UTR_core fragment were amplified [12,13] All amplified products were analyzed by the Sanger sequencing method and aligned by CLUSTALW program. A phylogenetic tree was constructed by neighbor-joining method and genetic distance was calculated by the Kimura distance formula (MEGA5 software, www.megasoftware.net).

Statistical analysis

Results were analyzed using SPSS version 20 (IBM). Differences were considered to be statistically significant if $P<0.05$. Patient characteristics were compared using the Chi-square test for categorical data and the Mann-Whitney U test and the Kruskal Wallis test for continuous data. Factors associated with the presence of HCV-antibody or HCV-RNA was identified using logistic regression models. The multivariate models included all variables that were significant at the $P<0.10$ level in the univariate analysis.

Results

Factors associated with HCV antibody status

A total of 426 from 619 (67%) HIV infected-individuals had antibodies against HCV (HCV-Ab). Most of our HIV-infected patients were young males with a history of IDU. Male gender and IDU were more often found in HCV Ab-positive patients ($P<0.001$). The period of injecting drug use was longer in HCV-Ab positive patients than in HCV-Ab negative patients (5 *vs.* 2 years, $P<0.001$), tattoo experiences were more often found in the HCV-Ab positive group (43% *vs.* 9%, $P<0.001$). More patients with HIV WHO stage 3 or 4 were (57% *vs.* 36%, $P<0.001$) and lower

median CD4 cells counts were found in the HCV-Ab positive patients than in HCV-Ab negative patients (198 cells/mm^3 *vs.* 207 cells/mm^3, *P*<0.001). However, lower HIV-RNA levels were found in HCV-Ab positive vs HCV-Ab negative patients (3.5 log^{10} copies/ml *vs.* 4.8 log^{10} copies/ml, *P*<0.001). HCV-Ab positive patients had a higher median liver enzymes levels (*P*<0.001).

More patients were taking HAART in the HCV-Ab positive group vs HCV-Ab negative patients (37% *vs.* 20%, *P*<0.001). To eliminate a potential effect of HIV treatment on liver disease status, further analyses were performed in 416 HIV-untreated participants. Similar trends were obtained; lower CD4 cells counts and lower HIV-RNA levels and higher liver enzymes levels were more often found in HCV-Ab positive patients vs HCV-Ab negative patients (Table 1). In multivariate analysis, four factors (male gender, age below 30 and a history of IDU or tattooing) were associated with HCV-Ab positivity (table 2). Additionally, in IDU drug use longer than five years was associated with an increased odd for HCV-Ab positivity. Interestingly, patients with HIV-RNA plasma level ≥10^5 copies/ml had decreased odds for being HCV-Ab positive.

Factors associated with chronic HCV infection

Of 416 HCV Ab-positive patients, 94 (22%) had insufficient sample volumes and could not be analyzed further. From the remaining 322 HCV Ab-positive patients 265 (82%) of them had detectable HCV-RNA. Logistic regression was applied to observe odds of having chronic HCV infection (Table 3). No differences were found in socio-demographics variables between chronic HCV and cleared HCV infection. In the multivariate model, patients with CD4 cells counts ≥50 cells/mm^3 had increased odds of having chronic HCV infection compared to patients with CD4 cells counts <50 cells/mm^3 (*P*=0.047 OR 2.01[95% CI 1.01-4.01]). The increased odd of having chronic HCV infection was also found in patients with increased ALT levels compared to those patients with normal ALT level (*P*=0.001 OR 2.91 [95% CI 1.41-5.99]). Finally, compared to HBsAg-negative patients, HBsAg-positive patients had a 75% decreased odd for chronic HCV infection (*P*=0.001 OR 0.25 [95% CI 0.08-0.78])

HCV variability and genotypic distribution

From 322 HCV Ab positive individuals plasma HCV-RNA was tested, 57 individuals did not have detectable HCV RNA, 82 individuals had a viral load $<10^5$ IU/ml, and 183 above $>10^5$ IU/ml. The HCV genotypes (based on two fragments 5'UTR_core and NS5B) was determined in samples with a HCV viral load $>10^5$ IU/ml. The results obtained with both fragments were concordant for all samples. The most prevalent sub genotype was 1a (50%), followed by 3k (16%), 1c (14%), 4a (9%), 3a (8%) and 1b (3%) (Figure1). The closest genetic distance was observed for sub genotypes 1a and 4a (genetic distance 0.008), followed by sub genotypes 3k (0.020), 1c (0.020), 1b (0.025) and 3a (0.026).

Factors associated with HCV genotype 1

The characteristics of patients infected by different HCV genotypes 1 (1a, 1b and 1c) and genotype 3 and 4 (3a, 3k, 4a) were compared. Common characteristics, except the median of age and the HCV-RNA levels, were found among patients with sub genotype 1, 3 and 4. Compared to individuals infected by HCV genotype 3, HCV-RNA levels were significantly lower in patients infected with HCV sub genotype 1 (median 6.3 $\log_{10}$ IU/ml 1 *vs.* 6.6 log10 IU/ml, *P*=0.001). Patients with HCV genotypes 4 were younger compared with patients with HCV sub genotypes 3 (median 25 years old *vs.* 30 years old, *P*=0.016).

Males had increased odds for having HCV genotype 1 infection compared to females (OR, 2.84 [95% CI, 1.16-6.97]; *P*=0.023). Patients with tattoo experiences had decreased odds of having HCV genotypes 1 infection compared with those patients without tattoo experiences (OR, 0.47 [95% CI, 0.24-0.89]; *P*=0.022).

Discussion

Our study describes the prevalence and risk factors for HCV infection in HIV-infected patients from West Java, Indonesia. We observed that 67% had been infected with HCV, reflected by the presence of HCV antibodies, of which 82% had a chronic infection. HCV infection was more often detected among individuals 1) of male gender, 2) young age (below 30 years), 3) a history of injecting drug use, especially if longer than 5 years and 4) a tattoo history. [15-17] The prevalence of HCV infection in our HIV cohort is substantially higher than found in the general

Indonesian population (3.9%), for whom contaminated blood products are the main risk factor.. [18] Different routes of HCV transmission play a role asking for different preventive strategies. First, needle/syringe exchange and opiate substitution programs may help to prevent both HIV and HCV transmission. [19] Second, policies to regulate and control tattoo parlors could help to reduce HCV transmission. [20] Third, awareness of transmission routes of HCV should be created among HIV infected patients. [21]

It has been documented that HIV infection can accelerate HCV disease progression [8, 22-24], however, our results suggest the possibility of a reverse correlation between HIV disease status and HCV infection. First, HCV infected patients had less HIV replication than those patients who never been infected by HCV. Second, chronically infected HCV patients had higher CD4 cells count than patients without detectable HCV-RNA but positive for HCV-antibody. An explanation could be that presence of multiple viral infections in our patient cohort affects the pathogenesis of both infections. A study by Tenckhoff et al., (2012) found that GBV-C infection in HCV/HIV infected hemophiliac patients was associated with lower HIV-RNA levels and higher CD4 cell counts .A high prevalence of GBV infection was also found in patients co-infected with HCV and HIV [25]. Further work is needed to clarify the relationship between GBV-C infection and disease progression in our cohort.

In this study, we demonstrated that the presence of serum HBsAg was negatively correlated with HCV-RNA positivity (OR, 0.271 [95% CI, 0.085-0.864]; P<0.001). This negative correlation may be explained by reciprocal inhibition occurring between HBV and HCV infection [26]. In dual HBV/HCV-infected patients, HBsAg was more often undetectable in patients with persistent HCV infection [27] and HCV-RNA was more often undetectable in patients with persistent HBsAg detection. [28] However, in HBV/HCV co-infected patients who reached undetectable HCV-RNA before HBV treatment was initiated, HBV suppression might not attribute to HCV rebound and the contrary might occur. [29] In HBV/HCV co-infected individuals, who reached undetectable HBsAg before HCV treatment was initiated, HCV suppression might attribute to HBV rebound. [26] In our cohort, all HBV infected patients were given lamivudine, but none of them were given HCV treatment. As we did not determine HCV-RNA before the start of HBV treatment we could not address the effect of HBV treatment on HCV-RNA levels.

In our study most HIV patients were infected with HCV sub genotype 1a, while in the general population, sub genotype 1b was found to predominate [30, 31]. Different treatment outcome between HCV sub genotype 1a and 1b have been demonstrated. Vispo et al., (2012) had shown that HCV sub genotype 1a had lower virological response to pegylated infeterferon (PEG + IFN) plus ribavirin therapy than HCV sub genotype 1b. [32] In addition, with the first generation of HCV protease inhibitors such as telaprevir , selection of drug resistant variants and viral breakthrough has been observed more frequently with sub genotype 1a than sub genotype 1b. [33, 34]

Genetic distance analysis, suggests that sub genotype 4a, in addition to 1a, was recently introduced among HIV infected patients in Indonesia. The HCV treatment efficacy of sub genotype 4a is limited to pegylated interferon and ribavarine therapy [35, 36], no information about the clinical efficacy of direct acting antivirals (DAA's) is available. Thus, our study further illustrates the problems we may face when treating HCV/HIV co-infected individuals in West Java.

This study had several limitations. First, acute HCV infection among HIV-infected patients could not be determined, because plasma HCV-RNA tests were limited to patients who were positive for HCV antibodies. Second, liver disease progression parameters other than ALT and AST were not assessed and the use of alcohol or hepatotoxic drugs was not taken into account. [37] Finally, only individuals with a high HCV plasma level were genotyped.. Despite these limitations, we believe that our study provides a comprehensive overview of the epidemiology of HCV in HIV-infected patients in West Java, Indonesia.

In summary, a high prevalence of chronic HCV infection was found in HIV infected patients. The large proportion of sub genotypes 1a will affect the success of HCV therapy.. Several routes of transmission contribute to the high prevalence of HCV co-infection requiring different preventive strategies

Acknowledgements

We thank the staff and patients of the Hasan Sadikin Hospital, West Java, Indonesia, for their participation and the Banceuy Narcotic Prison for hosting the cohort study of blood borne infections in their institution. We are grateful to Dept. of Virology, Erasmus Medical Center, the

Netherlands for donating reagents for sample testing and for additional training support in West Java. We acknowledge the financial support of the European Commission (Sixth Framework Programme; Specific Targeted Research Project no: 318/10150A – EC SANTE/2005/105-033).

Disclosure statement

None of the authors declares any conflict of interest.

References

1. Alter, M.J., *Epidemiology of viral hepatitis and HIV co-infection.* J Hepatol, 2006; **44**(1 Suppl): S6-9.
2. Sulkowski, M.S., *Viral hepatitis and HIV coinfection.* J Hepatol, 2008;**48**(2):353-67.
3. Thomas, D.L., et al., *The natural history of hepatitis C virus infection: host, viral, and environmental factors.* JAMA, 2000. **284**(4): p. 450-6.
4. Thomson, E.C., et al., *Delayed anti-HCV antibody response in HIV-positive men acutely infected with HCV.* AIDS, 2009. **23**(1): p. 89-93.
5. Price, J.C. and C.L. Thio, *Liver disease in the HIV-infected individual.* Clin Gastroenterol Hepatol, 2010. **8**(12): p. 1002-12.
6. UNAIDS, *UNAIDS global report 2010.* 2010.
7. Wisaksana, R., et al., *Response to first-line antiretroviral treatment among human immunodeficiency virus-infected patients with and without a history of injecting drug use in Indonesia.* Addiction, 2010. **105**(6): p. 1055-61.
8. Anggorowati, N., et al., *Clinical and virological characteristics of hepatitis B or C virus co-infection with HIV in Indonesian patients.* J Med Virol, 2012. **84**(6): p. 857-65.
9. Alisjahbana, B., et al., *Prevention, control and treatment of HIV-AIDS among injecting drug use in Bandung, Indonesia.* Acta Med Indones, 2009. **41 Suppl 1**: p. 65-9.
10. Utsumi, T., et al., *Detection of highly prevalent hepatitis B virus co-infection with HIV in Indonesia.* Hepatol Res, 2012.
11. Fibriani, A., et al., *Low cost HIV-1 quantitative RT-PCR assay in resource-limited settings: improvement and implementation.* J Virol Methods, 2012. **185**(1): p. 118-23.
12. Mellor, J., et al., *Investigation of the pattern of hepatitis C virus sequence diversity in different geographical regions: implications for virus classification. The International HCV Collaborative Study Group.* J Gen Virol, 1995. **76 (Pt 10)**: p. 2493-507.
13. Kageyama, S., et al., *A natural inter-genotypic (2b/1b) recombinant of hepatitis C virus in the Philippines.* J Med Virol, 2006. **78**(11): p. 1423-8.
14. Nijhuis, M., C.A. Boucher, and R. Schuurman, *Sensitive procedure for the amplification of HIV-1 RNA using a combined reverse-transcription and amplification reaction.* Biotechniques, 1995. **19**(2): p. 178-80, 182.
15. Nelwan, E.J., et al., *Indonesian prisons and HIV: part of the problem, part of the solution?* Acta Med Indones, 2009. **41 Suppl 1**: p. 52-6.
16. Miller, E.R., et al., *Markers and risk factors for HCV, HBV and HIV in a network of injecting drug users in Melbourne, Australia.* J Infect, 2009. **58**(5): p. 375-82.
17. Beyrer, C., et al., *Characterization of the emerging HIV type 1 and HCV epidemics among injecting drug users in Dushanbe, Tajikistan.* AIDS Res Hum Retroviruses, 2009. **25**(9): p. 853-60.

18. Akbar, N., et al., *Ethnicity, socioeconomic status, transfusions and risk of hepatitis B and hepatitis C infection.* J Gastroenterol Hepatol, 1997. **12**(11): p. 752-7.
19. Lee, K.C., W.W. Lim, and S.S. Lee, *High prevalence of HCV in a cohort of injectors on methadone substitution treatment.* J Clin Virol, 2008. **41**(4): p. 297-300.
20. Haley, R.W. and R.P. Fischer, *The tattooing paradox: are studies of acute hepatitis adequate to identify routes of transmission of subclinical hepatitis C infection?* Arch Intern Med, 2003. **163**(9): p. 1095-8.
21. Afriandi, I., et al., *HIV and injecting drug use in Indonesia: epidemiology and national response.* Acta Med Indones, 2009. **41 Suppl 1**: p. 75-8.
22. Soriano, V., A.S. Perelson, and F. Zoulim, *Why are there different dynamics in the selection of drug resistance in HIV and hepatitis B and C viruses?* J Antimicrob Chemother, 2008. **62**(1): p. 1-4.
23. Mehta, S.H., et al., *Protection against persistence of hepatitis C.* Lancet, 2002. **359**(9316): p. 1478-83.
24. Lin, W., et al., *HIV increases HCV replication in a TGF-beta1-dependent manner.* Gastroenterology, 2008. **134**(3): p. 803-11.
25. Anggorowati, N., et al., *GB virus C infection in Indonesian HIV-positive patients.* Microbiol Immunol, 2013. **57**(4): p. 298-308.
26. Liu, C.J., P.J. Chen, and D.S. Chen, *Dual chronic hepatitis B virus and hepatitis C virus infection.* Hepatol Int, 2009. **3**(4): p. 517-25.
27. Liaw, Y.F., *Hepatitis C virus superinfection in patients with chronic hepatitis B virus infection.* J Gastroenterol, 2002. **37 Suppl 13**: p. 65-8.
28. Pontisso, P., et al., *Hepatitis C genotypes in patients with dual hepatitis B and C virus infection.* J Med Virol, 1996. **48**(2): p. 157-60.
29. Chakvetadze, C., et al., *Reactivation of hepatitis B virus replication during peginterferon-ribavirin therapy in an HIV/hepatitis C virus-co-infected patient with isolated anti-hepatitis B core antibodies.* AIDS, 2007. **21**(3): p. 393-4.
30. Utama, A., et al., *Hepatitis C virus genotype in blood donors and associated liver disease in Indonesia.* Intervirology, 2008. **51**(6): p. 410-6.
31. Utama, A., et al., *Genotype diversity of hepatitis C virus (HCV) in HCV-associated liver disease patients in Indonesia.* Liver Int, 2010. **30**(8): p. 1152-60.
32. Vispo, E., et al., *Different impact of IL28B polymorphisms on response to peginterferon-alpha plus ribavirin in HIV-positive patients infected with HCV subtypes 1a or 1b.* J Clin Virol, 2012. **55**(1): p. 58-61.
33. Kieffer, T.L., et al., *Telaprevir and pegylated interferon-alpha-2a inhibit wild-type and resistant genotype 1 hepatitis C virus replication in patients.* Hepatology, 2007. **46**(3): p. 631-9.
34. Sarrazin, C., et al., *Dynamic hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir.* Gastroenterology, 2007. **132**(5): p. 1767-77.
35. Mira, J.A., et al., *Hepatitis C virus genotype 4 responds better to pegylated interferon with ribavirin than genotype 1 in HIV-infected patients.* AIDS, 2012. **26**(13): p. 1721-4.
36. Shaker, O.G. and N.A. Sadik, *Polymorphisms in interleukin-10 and interleukin-28B genes in Egyptian patients with chronic hepatitis C virus genotype 4 and their effect on the response to pegylated interferon/ribavirin-therapy.* J Gastroenterol Hepatol, 2012. **27**(12): p. 1842-9.
37. Iskandar, S., et al., *High risk behavior for HIV transmission among former injecting drug users: a survey from Indonesia.* BMC Public Health, 2010. **10**: p. 472.

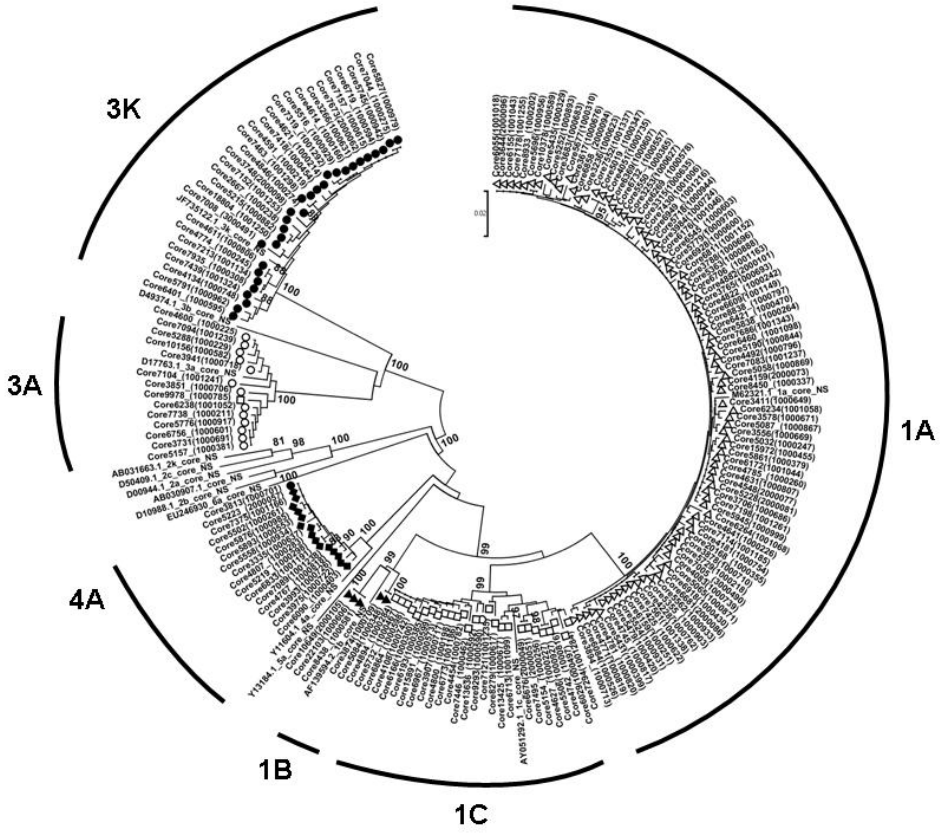

Figure 1 Phylogenetic analysis of 183 HCV sequences from HCV/HIV co-infected patients. The tree was constructed with neighbor-joining method based on combination of 5'ÚTR-Core and NS5b fragments (753 kb) of 14 HCV reference strains. Reference isolates are indicated with their accession numbers and sub types. The number inside the tree indicates the bootstrap reliability. The most prevalence genotype was 1a (△), followed by 3k (●), 1c (□), 3a (◯), 4a (◆) and 1b (▲).

Table 1 HIV and liver disease parameters in 416 untreated HIV patients by HCV-antibody status.

Characteristics	Total patients (N=416)	HCV-antibody positive (N=260)	HCV -antibody negative (N=156)	*P-value* [a]
HIV disease status				
Median CD4 count, cells/mm^3 [IQR]	110 [27-329]	85 [23-288]	193 [44-365]	**<0.001**
Median HIV-RNA, $\log_{10}$ copies/ml [IQR]	4.7 [3.9-5.3]	4.6 [3.5-5.2]	5 [4.4-5.6]	**<0.001**
Liver disease status				
Median ALT, IU/L [IQR]	28 [17-50]	34 [20-59]	14 [20-34]	**<0.001**
Median AST, IU/L [IQR]	31 [22-50]	35 [26-57]	25 [18-39]	**<0.001**

Table 2 Factors associated with detectable HCV-antibodies among 619 HIV infected individuals

	No. of patients	No. of HCV-antibody positive	No. of HCV-antibody negative	Univariate analysis		Multivariate analysis[a]	
	(%)	(%)	(%)	OR (95% CI)	*P-value*	OR (95% CI)	*P-value*
Total	619	416	203				
Socio-demographic							
Male gender	450 (73)	371 (89)	79 (39)	1.29 (8.51-19.67)	<0.001	6.59 (3.64-11.92)	**<0.001**
Age ≥30 years old	154 (25)	95 (23)	59 (29)	0.72 (0.46-0.942)	0.093	0.41 (0.22-0.75)	**0.004**
IDU (Yes)	388 (63)	357 (86)	31 (15)	33.00 (20.96-53.78)	<0.001	21.26 (11.79-38.33)	**<0.001**
Length of IDU ≥5 years[b]	189 (49)	184 (52)	5 (16)	5.33 (2.08-14.73)	0.001	4.82 (1.75-13.27)	**0.002**
Tattoo (Yes)	201 (32)	180 (43)	21 (10)	6.61 (4.04-10.81)	<0.001	2.33 (1.22-4.44)	**0.010**
HIV disease status							
CD4 ≥50 cells/mm^3	457 (74)	304 (73)	153 (75)	1.03 (0.69-1.53)	0.863	N.A.	N.A.
HIV-RNA ≥10^5 copies/ml	161 (26)	85 (20)	76 (37)	0.43 (0.29-0.62)	<0.001	0.43 (0.23-0.83)	**0.012**
Liver disease status							
ALT above normal[c]	197 (32)	160 (38)	37 (18)	2.80 (1.87-4.21)	<0.001	1.21 (0.58-2.51)	0.607
AST above normal[d]	214 (35)	166 (40)	48 (24)	2.14 (1.47-3.13)	<0.001	2.09 (1.01-4.37)	**0.047**
HBsAg positive	44 (7)	35 (8)	9 (4)	1.98 (0.93-4.20)	0.075	0.531 (0.21-1.38)	0.192
Under HIV treatment	203 (33)	156 (38)	47 (23)	1.99 (1.36-2.92)	<0.001	2.08 (1.05-4.15)	**0.037**

[a]Multivariate models included all variables that were significant at the *P*<0.10 level in univariate analysis.
[b]Applied for patients with a history of IDU (N=388)
[c]ALT normal for female: 5-38 IU/L; male: 10-50 IU/L.
[d]AST normal for female: 6-34IU/L; male: 8-40 IU/L.
Abbrevations: IDU, injecting drug use; ALT, alanine aminotransferase; AST, aspartate aminotransferase; N.A., not applicable.

Table 3 Factors associated with detectable plasma HCV-RNA in 322 HCV antibody positive HIV-infected individuals

	No. of patients	No. of HCV-RNA positive	No. of HCV-RNA negative	Univariate analysis		Multivariate analysis[a]	
	(%)	(%)	(%)	OR (95% CI)	*P-value*	OR (95% CI)	*P-value*
Total	322	265	57				
HIV disease status							
CD4 ≥50 cells/mm^3	234 (73)	201 (76)	33 (58)	2.36 (1.29-4.32)	0.005	2.01 (1.01-4.01)	**0.047**
HIV-RNA ≥10^5 copies/ml	68 (21)	49 (18)	19 (33)	0.45 (0.24-0.85)	0.014	0.67 (0.32-1.39)	0.282
Liver disease status							
ALT above normal[b]	123 (38)	111 (42)	12 (21)	2.70 (1.37-5.35)	0.004	2.91 (1.41-5.99)	**0.004**
AST above normal[c]	129 (40)	111 (42)	18 (32)	1.56 (0.85-2.87)	0.152	N.A.	N.A.
HBsAg positive	28 (9)	16 (6)	12 (21)	0.24 (0.11-0.54)	0.001	0.25 (0.08-0.78)	**0.017**

[a]Multivariate models included all variables that were significant at the *P*<0.10 level in univariate analysis.
[b]ALT normal for female: 5-38 IU/L; male: 10-50 IU/L.
[c]AST normal for female: 6-34IU/L; male: 8-40 IU/L.
Abbrevations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; N.A., not applicable.

Table a. Characteristics in relation to HCV-antibody status among 619 HIV infected patients (Supplement)

Characteristic	Total patients	HCV-antibody positive	HCV -antibody negative	*P-value* [a]
Number of patients	619	416	203	
Socio-demographics				
Gender, *N* (%)				
Male	450 (73)	371 (89)	79 (39)	**<0.001**
Female	169 (27)	45 (11)	124 (61)	
Median age, years [IQR]	29 (26-31)	29 (26-31)	28 (25-34)	0.620
History of IDU, *N* (%)				
No	184 (30)	22 (5)	162 (80)	
Yes	388 (63)	357 (86)	31 (15)	**<0.001**
Unknown	47 (7)	37 (9)	10 (5)	
Median length of IDU[b], years [IQR]	5 (3-8)	5 (3-8)	2 (1-4)	**<0.001**
Tattoo, *N* (%)				
No	342 (55)	185 (45)	157 (77)	
Yes	201 (32)	180 (43)	22 (11)	**<0.001**
Unknown	76 (12)	51 (12)	25 (12)	
HIV disease status				
WHO, *N* (%)				
Stage 1 or 2	125 (20)	48 (12)	77 (38)	
Stage 3 or 4	314 (51)	240 (58)	74 (36)	**<0.001**
Unknown	180 (29)	128 (30)	52 (26)	
Median CD4 count, cells/mm^3 [IQR]	205 (51-346)	198 (50-343)	207 (52-368)	**<0.001**
Median HIV-RNA, $\log_{10}$ copies/ml [IQR]	4.2 (<2.8-5.1)[c]	3.5 (<2.8-4.9)[c]	4.8 (<2.8-5.5)[c]	**<0.001**
Liver disease status				
Median ALT, IU/L [IQR]	33 (18-56)	38 (23-66)	20 (14-37)	**<0.001**
Median AST, IU/L [IQR]	32 (23-50)	35 (26-56)	24 (18-39)	**<0.001**
HBsAg positive, *N* (%)				
Negative	575 (93)	381 (92)	194 (96)	**0.007**
Positive	44 (7)	35 (8)	9 (4)	
HIV ART status				
Untreated, *N* (%)	416 (67)	260 (62)	156 (76)	**<0.001**
Treated, *N* (%)	203 (33)	156 (38)	47 (23)	
NRTI backbone of ARV therapy[d], *N* (%)				
Zidovudine + lamivudine	146 (72)	113 (72)	33 (70)	0.805
Stavudine + lamivudine	56 (27)	42 (27)	14 (30)	
Tenovofir + lamivudine	1 (1)	1 (1)	0 (0)	
NNRTI / PI component of ARV therapy[d], *N* (%)				
Nevirapine	131 (65)	97 (62)	34 (73)	0.361
Efavirenz	69 (34)	57 (37)	12 (25)	
Lopinavir + ritonavir	3 (1)	2 (1)	1 (2)	
Median treatment duration[d], months [IQR]	12 (22-31)	23 (14-32)	16 (19-25)	**0.006**

[a]Characteristics were compared between chronic HCV infected and viral negative patients using the Chi-square test for catergorical variables and the Mann-Whitney U test for countinuous variables. Differences were considered to be statistically significant if $P<0.05$ (bold font)
[b]Applied for patients with a history of IDU.
[c]Limit detection of HV-RNA test was 2.8 $\log_{10}$ copies/ml.
[d]Applied for patients under HIV treatment
Abbrevations: IQR, interquartile range; IDU, intravenous drug use; WHO, world health organization; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table b. Characteristics in relation to plasma HCV-RNA among 322 HCV antibody positive HIV infected individuals (Supplement)

Characteristic	Total patients	HCV-RNA positive	HCV -RNA negative	*P-value* [a]
Number of patients	322	265	57	
Socio-demographics				
Gender, *N* (%)				
Male	285 (88)	232 (87)	53 (93)	0.243
Female	37 (12)	33 (13)	4 (7)	
Median age, years [IQR]	29 (26-31)	29 (26-31)	29.5 (26.8-32)	0.270
History of IDU, *N* (%)				
No	19 (6)	16 (6)	3 (5)	
Yes	282 (87)	233 (88)	49 (86)	0.739
Unknown	21 (7)	16 (6)	5 (9)	
Median length of IDU[b], years [IQR]	5 (3-8)	5 (3-8)	4 (2-8)	0.328
Tattoo, *N* (%)				
No	154 (48)	132 (50)	22 (39)	
Yes	136 (42)	109 (41)	27 (47)	0.242
Unknown	32 (10)	24 (9)	8 (14)	
HIV disease status				
WHO, *N* (%)				
Stage 1 or 2	34 (10)	30 (11)	4 (7)	
Stage 3 or 4	189 (59)	155 (58)	34 (60)	0.611
Unknown	99 (31)	80 (31)	19 (33)	
Median CD4 count, cells/mm^3 [IQR]	196 (45-339)	206 (54-341)	71.5 (11-337)	**0.049**
Median HIV-RNA, $\log_{10}$ copies/ml [IQR]	3.5 (<2.8-4.9)[c]	3.2 (<2.8-4.8)[c]	4.4 (<2.8-5.4)[c]	**0.031**
Liver disease status				
Median ALT, IU/L [IQR]	39 (23-66)	42 (27-70)	25 (15-41)	**<0.001**
Median AST, IU/L [IQR]	35 (26-57)	36 (27-59)	27 (22-44)	**0.002**
HBsAg positive, *N* (%)				
Negative	294 (91)	249 (94)	45 (79)	**<0.001**
Positive	28 (9)	16 (6)	12 (21)	
HIV ART status				
Untreated, *N* (%)	200 (62)	161 (61)	39 (68)	0.279
Treated, *N* (%)	122 (38)	104 (39)	18 (32)	
NRTI backbone of ARV therapy[d], *N* (%)				
Zidovudine + lamivudine	88 (72)	77 (74)	11 (61)	
Stavudine + lamivudine	33 (27)	26 (25)	7 (39)	0.444
Tenovofir + lamivudine	1 (1)	1 (1)	0 (0)	
NNRTI / PI component of ARV therapy[d], *N* (%)				
Nevirapine	75 (61)	62 (60)	13 (72)	
Efavirenz	47 (38)	42 (39)	5 (28)	0.310
Lopinavir + ritonavir	1 (1)	1 (1)	0 (0)	
Median treatment duration[d], months [IQR]	23 (13-32)	23 (14-31)	24 (11-32)	0.851

[a]Characteristics were compared between chronic HCV infected and viral negative patients using the Chi-square test for catergorical variables and the Mann-Whitney U test for countinuous variables. Differences were considered to be statistically significant if $P<0.05$ (bold font)
[b]Applied for patients with a history of IDU.
[c]Limit detection of HV-RNA test was 2.8 $\log_{10}$ copies/ml.
Abbrevations: IQR, interquartile range; IDU, intravenous drug use; WHO, world health organization; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table c. Characteristics by HCV sub genotypes in plasma HCV-RNA positive HIV infected individuals (Supplement)

Characteristics	Total patients	Genotype 1	Genotype 3	Genotype 4	*P-value* [a]
Number of patients, *N*	183	124	43	16	
Socio-demographics					
Gender, *N* (%)					
Male	159 (87)	112 (90)	35 (81)	12 (75)	0.110
Female	24 (13)	12 (10)	8 (19)	4 (25)	
Median age, years [IQR]	29 (27-31)	28 (27-31)[A,B]	30 (28-31)[B]	25 (24-30)[A]	**0.016**[d]
History of IDU, *N* (%)					
No	10 (6)	8 (6)	1 (2)	1 (6)	
Yes	160 (87)	111 (90)	35 (82)	14 (88)	0.091
Unknown	13 (7)	5 (4)	7 (16)	1 (6)	
Tattoo, *N* (%)					
No	97 (53)	71 (58)	18 (42)	8 (50)	
Yes	77 (42)	40 (32)	20 (46)	8 (50)	0.219
Unknown	9 (5)	13 (10)	5 (12)	0 (0)	
HIV disease status					
Median CD4 count , cells/mm^3 [IQR]	218 (70-335)	201 (65-331)	212 (97-321)	262 (107-517)	0.318
Median HIV-RNA, $\log_{10}$ copies/ml [IQR]	<2.8 (<2.8-4.6)[b]	2.9 (<2.8-4.7)[b]	2.9 (<2.8-4.5)[b]	<2.8 (<2.8-4.8)[b]	0.735
Liver disease status					
Median ALT, IU/L [IQR]	39 (25-69)	39 (27-69)	41 (24-71)	33 (20-49)	0.495
Median AST, IU/L [IQR]	35 (27-59)	35 (27-60)	36 (27-49)	30 (24-39)	0.312
HBsAg positive, *N* (%)					
Negative	173 (94)	118 (95)	41 (95)	14 (88)	0.431
Positive	10 (6)	6 (5)	2 (5)	2 (12)	
Median HCV-RNA, $\log_{10}$ IU/ml [IQR]	6.3 (5.9-6.7)	6.3 (5.8-6.5)[A]	6.6 (6.2-7)[B]	6.6 (6.1-6.8)[A,B]	**0.001**[d]

[a]Characteristics were compared between chronic HCV infected and viral negative patients using the Chi-square test for catergorical variables and the Mann-Whitney U test for countinuous variables. Differences were considered to be statistically significant if $P<0.05$ (bold font)
[b]Applied for patients with a history of IDU.
[c]Limit detection of HV-RNA test was 2.8 $\log_{10}$ copies/ml.
Abbrevations: IQR, interquartile range; IDU, intravenous drug use; WHO, world health organization; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table d. Factors associated with HCV sub genotype 1 in 183 active HCV infected patients (Supplement)

	No. of patients	Genotype 1	Non-genotype 1	Univariate analysis		Multivariate analysis[a]	
	(%)	(%)	(%)	OR (95% CI)	*P-value*	OR (95% CI)	*P-value*
Total	183	124	59				
Socio-demographics							
Male gender	159 (87)	112 (90)	47 (80)	2.83 (0.99-5.69)	0.050	2.84 (1.16-6.97)	**0.023**
Age ≥30 years old	42 (23)	29 (23)	13 (22)	1.08 (0.51-2.27)	0.839	N.A.	N.A.
IDU (Yes)	160 (87)	111 (86)	49 (83)	1.74 (0.71-4.24)	0.221	N.A.	N.A.
Tattoo (Yes)	77 (42)	40 (32)	28 (47)	0.53 (0.28-0.99)	0.048	0.47 (0.24-0.89)	**0.022**
HIV disease status							
CD4 ≥50 cells/mm^3	143 (78)	92 (74)	51 (86)	0.65 (0.28-1.48)	0.302	N.A.	N.A.
HIV-RNA ≥10^5 copies/ml	72 (39)	49 (40)	23 (39)	1.43 (0.57-3.59)	0.449	N.A.	N.A.
Liver disease status							
ALT above normal[b]	72 (39)	48 (39)	24 (41)	0.98 (0.52-1.85)	0.945	N.A.	N.A.
AST above normal[c]	73 (40)	51 (41)	22 (37)	0.77 (0.40-1.45)	0.413	N.A.	N.A.
HBsAg positive	10 (5)	6 (5)	4 (7)	1.43 (0.39-5.27)	0.591	N.A.	N.A.

[a]Multivariate models included all variables that were significant at the *P*<0.10 level in univariate analysis.
[b]ALT normal for female: 5-38 IU/L; male: 10-50 IU/L.
[c]AST normal for female: 6-34IU/L; male: 8-40 IU/L.
Abbrevations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; N.A., not applicable.

Chapter 7

Summary
General discussion

Summary

Apart from two provinces of Papua and west Papua, the HIV epidemic in Indonesia is concentrated amongst key affected population resulting from a mix of two modes of transmissions, sexual transmission and drug injecting. From the time when the first case of AIDS were reported, which was in 1987, the number of new infections increased steadily and reached a total of 225 in 2000. Since then, the reported AIDS cases increased progressively fuelled by injecting drug user. According to Ministry of Health, the cumulative number of HIV infections in Indonesia has raised sharply from 7,195 in 2006 to 76,879 by 2011.[1] Integrated Bio Behavioral Surveillance on HIV (IBBS), a surveillance project among key populations which was carried out by the Ministry of Health and the Central Bureau of Statistics in 2007, reported a high HIV prevalence among Injecting Drug Users (52.4%).[2] To respond to this particular issue, the Minister of Health has initiated and supported harm reduction programs; specifically they increased the number of Needle and Syringe Program (NSP) and Methadone Maintenance Therapy (MMT) services in all provinces in Indonesia. The NSP increased from 120 in 2006 to 194 in 2011, in the same way, the number MMT services has increased from 11 in 2006 to 74 in 2011. [3] However, delivering HIV treatment and care among IDU might be more challenging than the prevention program itself, given that generally, HIV-IDU individuals are more likely to have more co-infection, specifically with hepatitis viruses.[4-6] This thesis focuses on HIV and hepatitis virus co-infection among HIV-IDU. Its findings may help to improve HIV treatment and care in resource limited countries, such as Indonesia.

Research findings

This thesis consist of three parts: the potential for using diagnostic tools to monitor HIV treatment, the outcome of first-line HIV treatment regimens and, the impact of HIV and hepatitis virus co-infection.

1. Characteristics of HIV patients in West Java, Indonesia

The majority of our recruited patients in West Java, Indonesia (**Chapter 2, 3, 5 and 6**) were relatively young males with a history of injecting drug use (IDU). Compared to the general population in Indonesia[7], HIV patients in West Java had higher social and economic statuses,

which is reflected by a higher percentage of patients who graduated from secondary school or university, the majority of whom also were gainfully employed. Clinically, most of the participants in our study had advanced HIV disease, with a high percentage of patients with WHO HIV disease stage 3 or 4 (>50% of patients) and CD4 cell counts <200 cells/mm^3 (>40% of patients). Twenty percent of patients were co-infected with tuberculosis (TB) and were taking rifampicin, ethambutol, pyrazinamide and isoniazid as part of their TB treatment regimen. Moreover, 7% of patients were co-infected with hepatitis B virus (HBV), and 62% were co-infected with hepatitis C virus (HCV). Despite these co-infection rates, the median liver enzyme levels were within their normal ranges, suggesting a low prevalence of liver inflammation among our patient cohort; nevertheless, these results are relatively preliminary and merit further study. In this setting, HIV treatment was initiated for patients with a CD4 cell count of <200 cells/mm^3 and/or were in WHO stage 3 or 4.[8] During the study period, two-thirds of the participants received highly active antiretroviral therapy (HAART), which consists of two nucleoside reverse transcriptase inhibitors (NRTIs; either zidovudine or stavudine, plus lamivudine) and a non-nucleoside reverse transcriptase inhibitor (NNRTI; either nevirapine or efavirenz). Viral load and CD4 cell counts were measured every six months in order to monitor the efficacy of HIV therapy.

2. HIV diagnosis

In **Chapter 2** we described the development and implementation of a low cost HIV viral load assay in Indonesian setting. In this study, we designed a novel RT-PCR quantitative assay which targets the HIV-1 *pol integrase* gene (INT). Subsequently, performances of the INT assay, in addition with a previously described Long Terminal Repeat (LTR) assay[9] and a combined INT/LTR dual target RT-PCR assay were compared. Among those three assays, the LTR-assay was found to be sensitive and cost-effective with the lowest coefficient of variation. Therefore, this LTR assay was further implemented in West Java, Indonesia. We observed that the LTR assay had excellent capability for monitoring treatment failure in clinical practice, with regard to its high positive and negative predictive value compared with other commercial assays.

3. HIV treatment outcome

Despite of the increasing number of antiretroviral therapy (ART) coverage in Indonesia within these few years, little is known about the treatment efficacy and the emergence of drug resistance mutations among HIV-infected patients in this country. Therefore in **Chapter 3**, we evaluated the virological response and the development of drug resistance during first-line ART in Indonesia where the majority of HIV-infected patients have a history of injecting drug use (IDU). In this study, we prospectively followed 575 patients who started ART between September 2007 and March 2010 in Hasan Sadikin Hospital Bandung. After six months of ART, a low virological failure rate was found among treated patients (9%). Of these failing patients 16 (47%) subjects had drug resistance mutations, predominantly lamivudine and NNRTIs associated mutations. Moreover, we found that a history of treatment discontinuation $\geq$1 month was strongly associated with virological failure; but a history of IDU was not.

In addition to lamivudine, emtricitabine is recommended in antiretroviral regimens for treating HIV-1. However, due to budget limitations, lamivudine, which is less expense, is more frequently used in first line regimens in resource limited countries, including Indonesia. Yet, the effectiveness of lamivudine compared to emtricitabine has not been extensively studied. Therefore In **Chapter 4**, we compared the efficacy between lamivudine- and emtricitabine-based regimens among HIV patients from Erasmus Medical Center. In this study, we included 196 HIV untreated patients who presented between 2002 and 2012 and who did not have any drug resistance mutations (DRM) at baseline. Compared with the emtricitabine group, patients in the lamivudine group experienced a higher rate of virological failure. Moreover, we found that lamivudine-based regimen was significantly associated with a shorter time to virological failure and more resistance mutations in reverse transcriptase gene. Consequently, our study suggests that a lamivudine-based regimen in first line ART has a lower efficacy than emtricitabine-based regimen.

4. HIV and hepatitis virus co-infection

As hepatitis B or C virus co-infection is common in our cohort, we studied the prevalence, risk factors, and genotypic distribution of hepatitis virus infection among HIV infected patients in West Java, Indonesia. In **Chapter** 5 we performed a cross sectional HBV study among a cohort of HIV

infected patients who presented in 2008 in Hasan Sadikin hospital, a referral hospital for West Java. A HBsAg test was performed in all recruited patients. Of 636 participants in this study, the rate of HBV co-infection was 7%. The proportion of males was higher in HBV/HIV co-infected patients than in HIV mono-infected patients. In addition, we found that a history of injecting drug use (IDU), but not tattooing, was associated with HBV co-infection. Further analyses using HIV and HBV treatment naive patients showed that low CD4 cells counts (<50 cells/mm^3), as well as high level of plasma HIV-RNA and AST were more often found in patients with active HBV replication. Finally, as in the general population, B3 was found to be the dominant subtype in HBV co-infected patients in our cohort.

Further investigation on HIV and HCV co-infection was reported in **Chapter 6.** In this study, HCV antibody tests were performed in 619 HIV-infected patients, subsequently HCV-RNA was determined in 322 HCV antibody positive HIV-infected patients. A high prevalence of HCV-antibody positive (67%) and active HCV infection (82%) was observed. Interestingly, we found a negative correlation between HIV infection and active HCV replication in our patients. As individuals with active HCV infection had lower HIV-RNA plasma levels than HCV RNA-negative individuals; furthermore, patients with low CD4 cells count (≥50 cells/mm^3) had higher chance of having active HCV infection as compared to those patient with a better immunological status. Remarkably, we also observed that HBsAg positive patients were less likely to have active HCV infection than HBsAg negative patients. Finally, we found that the most prevalent of HCV sub genotype in our cohort was 1a, followed by 3k, 1c, 4a, 3a and 1b.

References

1. AIDS national comission. Republic Indonesia country report on th efollow up to the delcraration of commitment on HIV/AIDS (UNGGAS) Report period 2010-2011; 2012.
2. AIDS national comission. The response to HIV and AIDS in Indonesia 2006-2011; 2011.
3. Ministry of health. Program Monitoring in Universal Access Report; 2011.
4. Thio CL. Hepatitis B and human immunodeficiency virus coinfection. Hepatology 2009;49(5 Suppl):S138-45.
5. Anggorowati N, Yano Y, Heriyanto DS, Rinonce HT, Utsumi T, Mulya DP, et al. Clinical and virological characteristics of hepatitis B or C virus co-infection with HIV in Indonesian patients. J Med Virol 2012;84(6):857-65.

6. Cescon A, Chan K, Raboud JM, Burchell AN, Forrest JI, Klein MB, et al. Significant differences in clinical outcomes between HIV-hepatitis C virus coinfected individuals with and without injection drug use history. AIDS 2014;28(1):121-7.
7. Statistik BP. Penduduk berumur 5 tahun keatas menurut wilayah dan pendidikan tertinggi yang ditamatkan 2010.
8. WHO. Antiretroviral therapy for HIV infection in adults and adolecents. 2006.
9. Drosten C, Panning M, Drexler JF, Hansel F, Pedroso C, Yeats J, et al. Ultrasensitive monitoring of HIV-1 viral load by a low-cost real-time reverse transcription-PCR assay with internal control for the 5' long terminal repeat domain. Clin Chem 2006;52(7):1258-66.

General discussion

Despite the recent significant decline in HIV prevalence in Asian countries, Indonesia is still experiencing an increase in new HIV infections, with a nearly three-fold increase in recent years. Despite this increase, the outcome of HIV treatment and our understanding of the impact of hepatitis B and/or C co-infection remains poorly understood. Therefore, in this thesis, the potential for using diagnostic tools to monitor HIV treatment and the outcome of first-line HIV treatment regimens was studied. Furthermore, the impact of HIV and hepatitis B and C virus co-infection was also investigated. The primary long-term goal of this research is to facilitate the delivery of improved HIV care, particularly in resource-limited countries such as Indonesia.

The most recent WHO guidelines recommend the use of plasma HIV RNA assays to monitor treated individuals. [10] However, due to the high costs associated with the use of commercial HIV-1 viral load tests, implementation in clinical practice remains a problem in resource-limited settings. Therefore, we developed and evaluated a low-cost in-house HIV-1 assay in our cohort (Chapter 2). Our analysis shows that the in-house assay is comparable to the commercial HIV-1 assay; specifically, both negative predictive value (NPV) and positive predictive value (PPV) were above 90%. Furthermore, the limit of detection (LOD) of the in-house assay satisfies the virological failure threshold of the 2011 National HIV Treatment Guideline (>5000 copies/ml)[14] and the 2013 WHO guideline (>1000 copies/ml).[13]

The next challenge is to facilitate the routine use of this affordable HIV viral load assay in other Indonesian provinces. Many laboratories in Indonesia lack the necessary infrastructure and knowledge to support the implementation. Therefore, at least two practical steps must be taken. First, for peripheral laboratories (which have limited facilities and knowledge base), sending the patient's sample to a central laboratory is a viable alternative. One option is to use the dried blood spot (DBS) method, in which the sample does not need to be shipped using complicated and expensive cooling conditions. Some studies have reported success using DBS with the commercial HIV-1 assay for monitoring HIV treatment in adult[15] and infant patients.[16] The feasibility and efficacy of using DBS combined with our in-house HIV-1 assay must be evaluated. The second necessary step is to transfer knowledge from central laboratories to the peripheral laboratories that currently have adequate infrastructure but lack the knowledge and skills needed

to implement our assay. We believe that the routine use of viral load assays to monitor HIV treatment in Indonesia can be achieved by establishing a solid collaboration and network among laboratories from both central and peripheral areas.

The majority of our patients in West Java, Indonesia were relatively young males with a history of injecting drug use (IDU). Compared to the general Indonesia population, the HIV patients in West Java have a higher social and economic status. This is evidenced by a higher percentage of patients who graduated from secondary school or university, the majority of whom also were gainfully employed.[1] Clinically, most of the participants in our study had advanced HIV disease, with a high percentage of patients with WHO HIV disease stage 3 or 4 (>50% of patients) and CD4 cell counts <200 cells/mm^3 (>40% of patients). Twenty percent of patients were co-infected with tuberculosis (TB) and were taking rifampicin, ethambutol, pyrazinamide and isoniazid as part of their TB treatment regimen. Moreover, 7% of patients were co-infected with hepatitis B virus (HBV), and 62% were co-infected with hepatitis C virus (HCV). Despite these co-infection rates, the median liver enzyme levels were within their normal ranges, suggesting a low prevalence of liver inflammation among our patient cohort. Nevertheless, these results are preliminary and merit further study. In our setting, HIV treatment was initiated in patients with a CD4 cell count of <200 cells/mm^3 and/or were in WHO stage 3 or 4.[2] During the study period, two-thirds of the participants received highly active antiretroviral therapy (HAART) which consists of two nucleoside reverse transcriptase inhibitors (NRTIs; either zidovudine or stavudine, plus lamivudine) and a non-nucleoside reverse transcriptase inhibitor (NNRTI; either nevirapine or efavirenz). Viral load and CD4 cell counts were measured every six months to monitor the efficacy of HIV therapy.

After receiving HAART for one year, the majority of patients had a good prognosis. Treatment failure, assessed by an intention to treat analysis, occurred in 166 out of 564 treated patients (29% of our cohort). Among these treated patients, virological failure, defined as plasma viral load >400 copies/ml after 6 months therapy, was found only in 40 patients (9% of our cohort) (Chapter 2). The rate of virological failure among IDU is similar to the rate among non-IDU (Chapter 3). In contrast, previous studies reported poorer virologic and immunologic outcomes to HAART in IDU compared to non-IDU.[14-17] This difference with other studies might

be explained by the comprehensive care program that we performed during our study period (IMPACT program, see chapters 3, 5 and 6). We propose that the comprehensive HIV care that was provided resulted in the retention of a larger proportion of IDU on effective treatment regimens. Similar findings were also reported from other studies.[18, 19] Consequently, withholding HAART among IDU in this setting is not justified.

On the other hand, the mortality rate within the first six months of treatment was high (11% of our cohort) and was associated with a low pre-treatment CD4 cell count (Chapter 3). Comparable studies were published from other HIV endemic regions such as Brazil, India and Africa.[20-22] There are two possible reasons for the low starting CD4 counts in our IDU cohort. In some countries—including Indonesia—IDU patients often have suboptimal access to HAART. Consequently, these patients often begin antiretroviral treatment only after they reach an advanced stage of HIV disease.[21] Second, a previous study from our cohort demonstrated that compared with non-IDU, IDU experienced a faster CD4 cell decline.[23] The faster CD4 decline found in our cohort might be explained by HCV co-infection, which occurred in the majority of our IDU patients (Chapter 6). It was previously reported that HCV/HIV co-infected patients generally have lower CD4 cell counts than patients with an HIV mono-infection[24]. This was indeed the case in our cohort, in which patients with HCV antibodies had lower CD4 cell counts (Chapter 6). The mechanism underlying CD4 cell depletion in HCV co-infected patients remains to be determined. HIV/HCV co-infection stimulates the expression of the cell death receptor Fas on $CD4^+$ T cells, leading to an increased apoptosis in this cell population.[25] Furthermore, it has been described that opioids—which are commonly used by IDU—can increase the expression of the HIV-1 co-receptor CCR5 and can stimulate HIV replication, thereby suppressing the immune system.[26, 27] Detecting HIV early in infection is therefore important, particularly among IDU patients.

HIV Test and Care (HTC) programs have been used widely throughout both developing and developed countries. Based on their operational sites, there are two types of HTC programs: facility-based HTC programs, in which testing and care are provided at the healthcare facility, and community-based HTC programs, in which the services are provided outside of the healthcare facility. A systematic review[11] revealed that community-based HTC is generally more successful than facility-based HTC in reaching patients early in the course of HIV infection. In

Indonesia, community-based HTC programs such as “Rumah Cemara” have been in operation since 2003. Given the increased success of identifying individuals earlier with community-based HTC, further implementation of this community-based approach in Indonesia may help to reduce HIV-related mortality and improve HIV treatment.

In our cohort, 53% of patients with virological failure to first line therapy had no drug-resistant mutations (DRMs). Furthermore, patients failing without DRM tended to have more HIV disease progression, as measured by higher viral loads and lower CD4 cell counts as compared to those with DRM. All patients with virological failure -with and without DRM- received adherence counseling, and the viral load test was repeated at a median of seven months after failure. In all failure patients without any DRM, viral suppression was observed, suggesting that extremely low compliance explained their initial treatment failure. Thus, our results highlight the importance of providing adherence intervention during therapy, particularly after first virological failure (Chapter 3).

Further analyses revealed that of those individuals failing with DRM, 88% had a lamivudine-associated mutation (Chapter 3). Our study based on the Erasmus MC cohort in the Netherlands showed higher efficacy of emtricitabine compared with lamivudine (Chapter 4). Replacing lamivudine with emtricitabine in first-line therapy might reduce the rate of virological failure and therefore may reduce the need to change to the more complex protease inhibitor-based treatment. Nevertheless, using emtricitabine as a first-line therapy is impractical in developing countries, given its high cost. Therefore, the cost-effectiveness of emtricitabine-based treatment regimens rather than lamivudine-based regimens must be evaluatedparticularly in countries such as Indonesia, which have limited access to first- and second-line antiretroviral therapies.

Regardless of differences in efficacy between emtricitabine or lamivudine use in first-line therapy, second-line therapy options in Indonesia are currently restricted to two NNRTIs and boosted protease inhibitor (PI) therapies. A previous study reported that a PI-based regimen (which combined protease inhibitors with zidovudine and lamivudine) yielded a significantly higher rate of virological suppression than protease inhibitors alone (95% vs. 80%, respectively; P=0.02).[17] Schoffelen et al. (2013) recently reported that a PI-based regimen yielded a good

virological response in patients who had failure on their first-line treatment with NRTIs and/or NNRTIs.[18] In their study, most of the patients received a combination of lamivudine, stavudine and nevirapine as their first-line therapy. After treatment failure, the second-line treatment combined recycled lamivudine with zidovudine and boosted lopinavir (LPV/r). Sustained virological suppression was achieved two years after the initiation of second-line treatment; 75% of patients had <400 copies/ml of plasma HIV RNA, and in 64% of the patients had plasma levels <50 copies/ml. Although the second-line PI-based regimens appear promising for treating patients who fail first-line therapy, the efficacy of these second-line regimens may unfortunately be limited in the future. A study in a cohort in Thailand found that the percentage of patients with virological suppression decreased significantly in each successive year of therapy.[19] Specifically, the percentage of patients with virological suppression was 80% after the first year of therapy, 75% after the second year, and then dropped to 66% after the third year of therapy. These data suggest that a proportion of patients who fail their second-line treatment will need third-line treatment, even in limited-resource settings. On the other hand, the third-line regimens currently recommended by the WHO generally exceed the budgetary possibilities in developing countries.[12] Therefore, preventing the failure of second-line therapy should be of high priority in such settings. One potential strategy is to improve therapy compliance using adherence intervention methods both in healthcare facilities and in the community. It has been showed that patients with poor adherence and patients who received a suboptimal second-line regimen would have a higher likelihood of second-line therapy failure.[20]

After the introduction of HAART, chronic liver disease is now the most common non-AIDS-related cause of death among HIV-infected patients. This high liver-related mortality rate in HIV-infected patients is due in part to co-infection with HCV and/or HBV. However, in developing countries the prevalence of liver-related death among these co-infected patients is generally underestimated. Limited access to diagnostic tool used to measure liver disease progression is one of the major reasons, and this was also the case during our study (Chapters 3, 5 and 6). Although liver biopsy is currently the gold standard for identifying and staging liver damage, performing liver biopsies in large cohort studies is not possible, particularly in regions with limited healthcare resources. Because liver biopsies are generally expensive, cause patient discomfort,

carry some risk and are difficult to repeat, non-invasive, affordable alternatives were developed. One such non-invasive tool for identifying liver fibrosis is the aspartate aminotransferase to platelet ratio index (APRI), which is measured as follows: [100 x (AST/ULN-AST)/platelet count], with AST and ULN-AST measured in IU/L and platelet count measured in 10^9/L. Previous studies reported that APRI can be used to assess liver fibrosis in HBV patients as well as in HCV-infected individuals.[21, 22] Compared with patients infected solely with HIV, HBV or HCV, patients who were-co-infected with either HIV/HBV or HIV/HCV had higher median APRI values.[23] In our study, we did not use APRI to measure liver inflammation in our HIV/hepatitis co-infected patients. Nevertheless, we found a significant correlation between patients with HIV/hepatitis co-infection and high AST levels. In Chapter 6, we report that the majority of HCV antibody-positive patients had higher AST levels compared to HCV antibody-negative patients. Hence, as reported in Chapter 5, high AST levels were also more prevalent among our patients with active HBV replication (HBV-DNA ≥20,000 IU/ml) than among patients with low HBV replication. Thus, it is suggested that APRI may also be used for monitoring liver inflammation in our cohort. Nevertheless, further work needs to be performed to evaluate this non-invasive diagnostic tool.

Our study reported in Chapter 5 was performed in 2008. At that time, we used the 2007 national HIV treatment guidelines, which still recommended the use of lamivudine in combination with either zidovudine or stavudine and either nevirapine or efavirenz as first-line therapy for treating HIV patients with or without HBV co-infection. Lamivudine, a nucleoside inhibitor with dual activity against both HIV and HBV, is often prescribed for treating HIV/HBV co-infected patients. On the other hand, although it is highly efficacious in treating HIV infections, lamivudine has a low genetic barrier for selecting drug-resistant mutations in HBV. A previous study reported that the prevalence of lamivudine-resistant HBV was 50% in HIV/HBV co-infected patients who received <24 months of lamivudine monotherapy, and this prevalence increased to 94% among HIV/HBV co-infected patients after >48 months of lamivudine monotherapy.[24] In our study (see Chapter 5), 45 of the 636 HIV-infected patients (7%) were positive for the hepatitis B surface antigen (HBsAg). Among these 45 HBsAg-positive patients, 29 were not treated for HIV, while the other 26 patients were treated for HIV (with lamivudine). No lamivudine-resistant mutations

were detected in the HIV-untreated patients. In contrast, three of the 26 HIV-treated patients had lamivudine-resistant mutations.

In 2011 the national guideline was changed: instead of lamivudine monotherapy, the new guideline recommends tenofovir combined with either lamivudine or emtricitabine for treating HIV/HBV co-infected patients. Consequently, managing patients who developed lamivudine-resistant mutations as a result of treatment under the previous guidelines is challenging. Previous studies demonstrated that tenofovir is effective at reducing HBV-DNA levels in HIV/HBV co-infected patients who carry either the wild-type or lamivudine-resistant virus.[25-27] Moreover, mutations causing resistance to tenofovir were not detected following long-term tenofovir therapy (42 months) in HIV/HBV co-infected patients.[28] Thus, tenofovir may be feasible for use in treating patients with HBV lamivudine-resistant mutations. However, HBV DNA in treated HBV/HIV co-infected patients should be monitored routinely to reduce the emergence of drug-resistant mutations that could reduce the efficacy of current treatment and limit future therapeutic options due to cross-resistance.

More than half of the participants in our study were infected previously with HCV, reflected by the high prevalence of detectable HCV antibodies in this cohort (Chapter 6). Despite the inverse correlation between HIV status and active HCV replication, the majority of HCV antibody-positive patients had advanced HIV disease. The advanced development of HIV infection in HCV antibody-positive patients was reflected by a higher percentage of patients in WHO HIV stage 3 or 4. In addition, the HCV antibody-positive patients had a lower median CD4 cell count. Finally, the HCV antibody-positive patients had significantly elevated liver enzyme levels, suggesting that patients who were infected with HCV have more active liver damage compared with patients who were never infected with HCV. Thus, our study is in agreement with other studies[29-33] in showing that the impact of HIV/HCV co-infection in HIV and liver disease progression is more intricate than the impact of HIV mono-infection alone. Treating HCV infection should therefore be considered in HIV/HCV co-infected patients. However, HCV treatments such as pegylated interferon and ribavirin, as well as the new generation of protease inhibitors, are extremely expensive. This financial limitation likely restricts the ability of affected patients to

access HCV treatment. Consequently, it is important to make affordable generic HCV drugs available, particularly to patients in low- and middle-income countries.

Overall conclusions

In this thesis, we reviewed the impact of HIV and HCV/HBV co-infection. We found that early HIV diagnosis is important to reduce HIV-related mortality and improve HIV treatment outcomes, particularly in key affected populations such as IDUs. Implementation of the highly sensitive viral load assay for monitoring HIV therapy can be achieved even in resource limited countries. Adherence interventions may be a crucial factor for maintaining patient compliance and reducing development of drug resistance viruses that can hamper further therapeutic options. The high prevalence of HIV and hepatitis co-infection emphasizes the need to routinely assess liver inflammation in order to reduce the number of liver-related deaths among HIV infected patients. Finally, the high rate of active HCV replication that was observed in our patients emphasizes the urgent need to provide affordable drugs to treat HCV in these patients.

Future studies are needed to improve the outcomes of patients co-infected with HIV and hepatitis. The impact of hepatitis co-infection on HIV treatment response in this cohort remains to be determined. Other high-risk groups such as sex workers and men who have sex with men (MSM) need to be included in these kinds of studies, and more attention should be given to virological monitoring, particularly in infants.

References

1. Statistik BP. Penduduk berumur 5 tahun keatas menurut wilayah dan pendidikan tertinggi yang ditamatkan In; 2010.
2. WHO. Antiretroviral therapy for HIV infection in adults and adolecents. 2006.
3. Wisaksana R, Indrati AK, Fibriani A, Rogayah E, Sudjana P, Djajakusumah TS, et al. Response to first-line antiretroviral treatment among human immunodeficiency virus-infected patients with and without a history of injecting drug use in Indonesia. Addiction 2010;105(6):1055-61.
4. Gupta RK, Hill A, Sawyer AW, Cozzi-Lepri A, von Wyl V, Yerly S, et al. Virological monitoring and resistance to first-line highly active antiretroviral therapy in adults infected with HIV-1 treated under WHO guidelines: a systematic review and meta-analysis. Lancet Infect Dis 2009;9(7):409-17.

5. Grigoryan A, Hall HI, Durant T, Wei X. Late HIV diagnosis and determinants of progression to AIDS or death after HIV diagnosis among injection drug users, 33 US States, 1996-2004. PLoS One 2009;4(2):e4445.
6. Brunetta DM, De Santis GC, Vilar FC, Brandao RA, Muniz RZ, de Lima GM, et al. Hematological particularities and co-infections in injected drug users with AIDS. Braz J Infect Dis 2013;17(6):654-6.
7. Orsetti E, Staffolani S, Gesuita R, De Iaco G, Marchionni E, Brescini L, et al. Changing characteristics and risk factors of patients with and without incident HCV infection among HIV-infected individuals. Infection 2013;41(5):987-90.
8. Korner C, Tolksdorf F, Riesner K, Kramer B, Schulte D, Nattermann J, et al. Hepatitis C coinfection enhances sensitization of CD4(+) T-cells towards Fas-induced apoptosis in viraemic and HAART-controlled HIV-1-positive patients. Antivir Ther 2011;16(7):1047-55.
9. Mahajan SD, Schwartz SA, Shanahan TC, Chawda RP, Nair MP. Morphine regulates gene expression of alpha- and beta-chemokines and their receptors on astroglial cells via the opioid mu receptor. J Immunol 2002;169(7):3589-99.
10. Steele AD, Henderson EE, Rogers TJ. Mu-opioid modulation of HIV-1 coreceptor expression and HIV-1 replication. Virology 2003;309(1):99-107.
11. Suthar AB, Ford N, Bachanas PJ, Wong VJ, Rajan JS, Saltzman AK, et al. Towards universal voluntary HIV testing and counselling: a systematic review and meta-analysis of community-based approaches. PLoS Med 2013;10(8):e1001496.
12. WHO. Antiretroviral therapy for HIV infection in adults and adolescents Recommendations for a public health approach 2010 revision. 2010.
13. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection recommendations for public health approach. Kuala Lumpur, Malaysia; 2013.
14. lingkungan KkRIDJppdp. Pedoman nasional tatalaksana klinis infeksi HIV dan terapi antiretroviral pada orang dewasa. Jakarta, Indonesia: Kementrian kesehatan Republik Indonesia Direktorat Jendral pengendalian penyakit dan penyehatan lingkungan 2011.
15. Kane CT, Ndiaye HD, Diallo S, Ndiaye I, Wade AS, Diaw PA, et al. Quantitation of HIV-1 RNA in dried blood spots by the real-time NucliSENS EasyQ HIV-1 assay in Senegal. J Virol Methods 2008;148(1-2):291-5.
16. Leelawiwat W, Young NL, Chaowanachan T, Ou CY, Culnane M, Vanprapa N, et al. Dried blood spots for the diagnosis and quantitation of HIV-1: stability studies and evaluation of sensitivity and specificity for the diagnosis of infant HIV-1 infection in Thailand. J Virol Methods 2009;155(2):109-17.
17. Delfraissy JF, Flandre P, Delaugerre C, Ghosn J, Horban A, Girard PM, et al. Lopinavir/ritonavir monotherapy or plus zidovudine and lamivudine in antiretroviral-naive HIV-infected patients. AIDS 2008;22(3):385-93.
18. Schoffelen AF, Wensing AM, Tempelman HA, Geelen SP, Hoepelman AI, Barth RE. Sustained virological response on second-line antiretroviral therapy following virological failure in HIV-infected patients in rural South Africa. PLoS One 2013;8(3):e58526.
19. Manosuthi W, Thongyen S, Nilkamhang S, Manosuthi S, Sungkanuparph S. Long-term treatment outcomes of ritonavir-boosted lopinavir monotherapy among HIV-infected patients who experienced NRTI and NNRTI failure. AIDS Res Ther 2012;9(1):8.
20. Pujades-Rodriguez M, Balkan S, Arnould L, Brinkhof MA, Calmy A, MSF AWGo. Treatment failure and mortality factors in patients receiving second-line HIV therapy in resource-limited countries. JAMA 2010;304(3):303-12.
21. Hung HH, Su CW, Lai CR, Chau GY, Chan CC, Huang YH, et al. Fibrosis and AST to platelet ratio index predict post-operative prognosis for solitary small hepatitis B-related hepatocellular carcinoma. Hepatol Int 2010;4(4):691-9.
22. Shin WG, Park SH, Jang MK, Hahn TH, Kim JB, Lee MS, et al. Aspartate aminotransferase to platelet ratio index (APRI) can predict liver fibrosis in chronic hepatitis B. Dig Liver Dis 2008;40(4):267-74.
23. Price JC, Seaberg EC, Badri S, Witt MD, D'Acunto K, Thio CL. HIV monoinfection is associated with increased aspartate aminotransferase-to-platelet ratio index, a surrogate marker for hepatic fibrosis. J Infect Dis 2012;205(6):1005-13.

24. Matthews GV, Bartholomeusz A, Locarnini S, Ayres A, Sasaduesz J, Seaberg E, et al. Characteristics of drug resistant HBV in an international collaborative study of HIV-HBV-infected individuals on extended lamivudine therapy. AIDS 2006;20(6):863-70.
25. Nunez M, Perez-Olmeda M, Diaz B, Rios P, Gonzalez-Lahoz J, Soriano V. Activity of tenofovir on hepatitis B virus replication in HIV-co-infected patients failing or partially responding to lamivudine. AIDS 2002;16(17):2352-4.
26. Stephan C, Berger A, Carlebach A, Lutz T, Bickel M, Klauke S, et al. Impact of tenofovir-containing antiretroviral therapy on chronic hepatitis B in a cohort co-infected with human immunodeficiency virus. J Antimicrob Chemother 2005;56(6):1087-93.
27. Nelson M, Portsmouth S, Stebbing J, Atkins M, Barr A, Matthews G, et al. An open-label study of tenofovir in HIV-1 and Hepatitis B virus co-infected individuals. AIDS 2003;17(1):F7-10.
28. Berg T, Zoulim F, Moeller B, Trinh H, Marcellin P, Chan S, et al. Long-term efficacy and safety of emtricitabine plus tenofovir DF vs tenofovir DF monotherapy in adefovir-experienced chronic hepatitis B patients. J Hepatol 2013.
29. Soriano V, Puoti M, Sulkowski M, Cargnel A, Benhamou Y, Peters M, et al. Care of patients coinfected with HIV and hepatitis C virus: 2007 updated recommendations from the HCV-HIV International Panel. AIDS 2007;21(9):1073-89.
30. Liu J, Chen X, Xie Q, Zhang W, Wei L, Shen T, et al. Drug resistance and HCV coinfection in former blood donors infected with HIV type 1 in China. AIDS Res Hum Retroviruses 2011;27(8):925-30.
31. Beyrer C, Patel Z, Stachowiak JA, Tishkova FK, Stibich MA, Eyzaguirre LM, et al. Characterization of the emerging HIV type 1 and HCV epidemics among injecting drug users in Dushanbe, Tajikistan. AIDS Res Hum Retroviruses 2009;25(9):853-60.
32. Glassner A, Eisenhardt M, Kokordelis P, Kramer B, Wolter F, Nischalke HD, et al. Impaired CD4(+) T cell stimulation of NK cell anti-fibrotic activity may contribute to accelerated liver fibrosis progression in HIV/HCV patients. J Hepatol 2013;59(3):427-33.
33. Nunez M, Soriano V. Hepatitis C virus (HCV) genotypes and disease progression in HIV/HCV-coinfected patients. J Infect Dis 2005;191(1):1-3.

Chapter 8

Nederlandse Samenvatting

Samenvatting

De voornaamste overdracht voor HIV in Indonesië vindt plaats via seksuele overdracht of intraveneus drugsgebruik. Sinds de eerst gerapporteerde aids casus in 1987 is het aantal nieuwe infecties in Indonesië continu gestegen, In eerste instantie tot het jaar 2000 nog geleidelijk tot 225 gevallen, na het millennium groeide het aantal gerapporteerde AIDS gevallen echter fors, mede aangewakkerd door een toename van infecties bij intraveneuze drugsgebruikers. Volgens het ministerie van volksgezondheid steeg het aantal HIV infecties van 7,195 in 2006 tot 76,897 in 2011. Geïntegreerde bio-gedrag surveillance (IBBS), een monitoringsproject onder risicogroepen uitgevoerd door het Ministerie van Volksgezondheid en het Centraal Bureau voor de Statistiek, rapporteerde in 2007 over een hoge HIV-prevalentie onder intraveneuze drugsgebruikers (IDG's) (52.4%). In een reactie hierop is het Ministerie van Volksgezondheid begonnen met het initiëren en ondersteunen van leedverzachtende initiatieven, in het bijzonder het uitbreiden van zogenaamde Naald en Spuitprogramma's (NSP) en therapeutische methadonservices (TMS) in alle provincies in Indonesië. Zo steeg het aantal NSP's van 120 in 2006 tot 194 in 2011. In diezelfde periode steeg het aantal MMT services van 11 tot 74. [3] Het leveren van effectieve behandeling en van HIV therapie aan IDG's is misschien nog wel een grotere uitdaging. Een van de oorzaken hiervoor is het hoge aantal co-infecties met hepatitisvirussen.[4-6] Dit proefschrift concentreert zich op HIV en hepatitis co-infecties onder HIV-geïnfecteerde IDG's in Indonesië. De beschreven bevindingen leveren een bijdrage aan verbeterde behandeling en verzorging van HIV-geïnfecteerde patiënten in Indonesië en andere derde wereld landen.

Samenvatting van de onderzoeksresultaten

Dit proefschrift bestaat uit drie delen: In het eerste deel wordt het potentieel van het gebruik aan diagnostische technieken voor HIV monitoring beschreven. Daarnaast worden de uitkomsten van eerstelijns HIV therapie in Indonesië beschreven. In het laatste deel wordt de impact van HIV- en hepatitisvirus co-infecties beschreven.

De meerderheid van onze geïncludeerde patiënten in West Java (**hoofdstukken 2, 3, 5 en 6**) waren relatief jonge drugsverslaafde mannen. Deze mannen hadden een hoger sociaal en economische status dan het landelijke gemiddelde. Dit wordt weerspiegeld door het hoge percentage patiënten met een middelbare school of universiteitsdiploma. Ook had de meerderheid van de patiënten een baan. Veelal presenteerde deze patiënten zich met HIV-symptomen in een ver gevorderd stadium (>50%, WHO stadium 3 tot 4) waarbij CD4+ cel aantallen lager waren dan 200 cellen/mm^3 (>40%). Zeven procent van de patiënten had een hepatitis B virus (HBV) co-infectie en 62% had een co-infectie met hepatitis C virus (HCV). Daarnaast had 20% procent van de patiënten tuberculose (TB). Zij werden daarvoor behandeld met rifampicine, ethambutol, pyrazinamide en isoniazide als onderdeel van het TB-behandelingsregime. Ondanks het hoge percentage hepatitis co-infecties lagen de leverenzymwaarden in de normale range. Dit suggereert een lage frequentie patiënten met een actieve leverontsteking in dit HIV-cohort. Deze voorlopige resultaten moeten echter nog verder worden onderzocht.

Voor patiënten in deze setting werd HIV-therapie gestart wanneer CD4 cel aantallen lager waren dan 200 cellen per mm^3 en/of wanneer zij zich presenteerden met AIDS in stadium 3 of 4 (WHO schaal). Tijdens de studieperiode werd aan twee derde van de patiënten *highly active anti-retroviral therapy* (HAART) voorgeschreven. Deze bestond uit een of twee nucleoside reverse-transcriptase remmers (NRTIs: zidovudine of stavudine en lamivudine) en een non-nucleoside reverse-transcriptase remmer (NNRTI; nevirapine of efavirenz). De virale load en CD4 cel aantallen werden elke zes maanden gemeten om de effectiviteit van deze therapie vast te stellen.

5. HIV diagnose

In **hoofdstuk 2** is de ontwikkeling en implementatie beschreven van een goedkope HIV virale lading test in Indonesië. Deze kwantitatieve test gebaseerd op reverse-transcriptase polymerase kettingreactie (RT-PCR) is gericht op het HIV-1 *ntegrase* gen (INT). De prestaties van deze test zijn vervolgens vergeleken met een vergelijkbare, eerder beschreven test gericht op het *HIV-1 long terminal repeat* (LTR) en de gecombineerde INT/LTR test. Uit deze vergelijking bleek de

LTR-test het meest gevoelig, rendabel en robuust te zijn. Daarom werd besloten de LTR-test te implementeren op West Java. De LTR-test bleek van uitstekende waarde voor het vaststellen van het falen onder therapie met hoge positieve en negatieve voorspellende waardes in vergelijking tot een commercieel verkrijgbare test.

6. Uitkomst van HIV therapie

De beschikbaarheid van HAART is in de afgelopen jaren toegenomen in Indonesië. Er is echter weinig bekend over de effectiviteit van deze therapie en het ontstaan van antiviraal resistente virussen onder deze HIV-geïnfecteerde patiënten. In **hoofdstuk 3** is daarom de virologische respons en ontwikkeling van antivirale resistentie geëvalueerd onder eerstelijns ART-therapie in Indonesië, waarbij de meerderheid van HIV-geïnfecteerde IDG patiënten zijn. Tijdens dit onderzoek werden 575 patiënten prospectief gevolgd. Allen startten ART-therapie tussen september 2007 en maart 2010 en waren onder behandeling in het Hasan Sadikin ziekenhuis in Bandung. Dit is een **tweede lijns** ziekenhuis voor West-Java. Na zes maanden ART-therapie bleek het aantal patiënten dat faalde onder therapie laag (9%). Onder de falende patiënten kon in 16 (47%) van hen een antiviraal resistent virus worden gedetecteerd. Veelal waren dit mutaties geassocieerd met lamivudine en NNRTIs antivirale resistentie. Daarnaast was het onderbreken van therapie langer dan een maand geassocieerd met virologisch falen; een historie van IDG was hiermee niet geassocieerd.

Naast lamivudine is emtricitabine aanbevolen in antiretrovirale regimes voor de behandeling van HIV-1 infecties. Omdat lamivudine goedkoper is wordt dit middel vaker gebruikt als onderdeel van eerstelijns regimes in derdewereldlanden, zoals in Indonesië. Het verschil in effectiviteit tussen lamivudine en emtricitabine is echter niet uitgebreid bestudeerd. In **hoofdstuk 4** is daarom een vergelijking gemaakt tussen de effectiviteit van regimes met lamivudine- en emtricitabine. In deze studie werden 196 onbehandelde HIV-patiënten geïncludeerd, die zich presenteerden tussen 2002 en 2012 in het Erasmus Medisch Centrum. Geen van deze patiënten was geïnfecteerd met een antiviraal resistent virus . Vergeleken met de emtricitabine behandelde groep was er in de lamivudine behandelde groep meer virologisch falen. Daarnaast kon het regime met lamivudine geassocieerd worden met een kortere tijd tot virologisch falen en werden

er in deze groep meer antivirale resistentiemutaties gevonden in het *reverse transcriptase* gen. Dit suggereert dat in eerstelijns ART-therapie regimes met lamivudine minder effectief zijn dan regimes gebaseerd op emtricitabine.

7. HIV en hepatitis co-infecties

Omdat co-infecties van HIV-patiënten met hepatitis B en C virus algemeen waren in het bestudeerde cohort zijn het voorkomen, de risicofactoren en genotype van hepatitis onder HIV-geïnfecteerden op West-Java verder in kaart gebracht. In **hoofdstuk 5** van dit proefschrift is een cross-sectionele HBV studie beschreven voor een cohort van HIV-geïnfecteerde patiënten die zich in 2008 in het Hasan Sadikin ziekenhuis presenteerden. Van de 636 geïncludeerde patiënten, allen HBsAg getest, was 7% positief. Het aantal mannelijke patiënten was hoger in de HBV-positieve groep vergeleken met de patiënten die geen co-infectie hadden. Een historie van intraveneus drugsgebruik was geassocieerd met een HBV co-infectie. Het laten zetten van een tatoeage was dit echter niet. Verdere analyse toonde aan dat lage CD4 cel aantallen (< 50 cellen per mm^3) en hoge plasma waarden voor HIV-RNA en AST vaker voorkwamen bij patiënten met actieve HBV replicatie. Tenslotte, HBV genotype B3 was het meest voorkomende genotype in het cohort van HBV geïnfecteerde patiënten met HIV.

Het onderzoek naar HIV en HCV co-infecties binnen het cohort is beschreven in **hoofdstuk 6**. In de hierin beschreven studie zijn HCV serologische tests uitgevoerd voor 619 HCV-geïnfecteerde patiënten en is het HCV-RNA bepaald in 322 HCV seropositieve HIV-geïnfecteerde patiënten. Er werd een hoge prevalentie van HCV-seropositieven (67%) hiervan had 82% een actieve HCV infecties . Een negatieve correlatie werd gevonden tussen HIV infectie en actieve HCV replicatie in de geselecteerde patiënten voor deze studie. Individuen met een actieve HCV infectie hadden namelijk een lagere HIV-RNA plasma waarden dan HCV-RNA negatieve individuen. Verder was de kans op een actieve HCV infectie groter in patiënten met lage CD4 cel aantallen ≤ 50 cellen per mm^3) vergeleken met patiënten in een betere immunologische conditie. Opmerkelijk was de observatie dat bij HBsAg-positieve patiënten de kans kleiner was op het vinden van een actieve

HCV infectie dan in HBsAg- negatieve patiënten. De meest voorkomende HCV sub-genotype in het cohort was van het 1a, gevolgde door het 3k, 1c, 4a en tenslotte het 1b genotype.

Referenties

1. AIDS national comission. Republic Indonesia country report on th efollow up to the delcraration of commitment on HIV/AIDS (UNGGAS) Report period 2010-2011; 2012.
2. AIDS national comission. The response to HIV and AIDS in Indonesia 2006-2011; 2011.
3. Ministry of health. Program Monitoring in Universal Access Report; 2011.
4. Thio CL. Hepatitis B and human immunodeficiency virus coinfection. Hepatology 2009;49(5 Suppl):S138-45.
5. Anggorowati N, Yano Y, Heriyanto DS, Rinonce HT, Utsumi T, Mulya DP, et al. Clinical and virological characteristics of hepatitis B or C virus co-infection with HIV in Indonesian patients. J Med Virol 2012;84(6):857-65.
6. Cescon A, Chan K, Raboud JM, Burchell AN, Forrest JI, Klein MB, et al. Significant differences in clinical outcomes between HIV-hepatitis C virus coinfected individuals with and without injection drug use history. AIDS 2014;28(1):121-7.
7. Statistik BP. Penduduk berumur 5 tahun keatas menurut wilayah dan pendidikan tertinggi yang ditamatkan 2010.
8. WHO. Antiretroviral therapy for HIV infection in adults and adolecents. 2006.
9. Drosten C, Panning M, Drexler JF, Hansel F, Pedroso C, Yeats J, et al. Ultrasensitive monitoring of HIV-1 viral load by a low-cost real-time reverse transcription-PCR test with internal control for the 5' long terminal repeat domain. Clin Chem 2006;52(7):1258-66.

Chapter 9

Curriculum Vitae

PhD portfolio

List of publications

Curriculum Vitae

Azzania Fibriani was born on December 5th, 1977, in Bandung, Indonesia. She obtained her bachelor's degree from Institut Teknologi Bandung, Indonesia in 2002. Prior to her bachelor degree, she awarded Japan International Cooperation Agency (JICA) scholarship and participated in Young Scientist Exchange Program (YSEP), a one year student exchanged program between Institut Teknologi Bandung, Indonesia and Tokyo Institute of Technology, Japan. During this program she conducted research in the molecular evolution of trappins genes, a family of secreted proteins composed of a transglutaminase substrate (TGS) domain and a whey acidic protein (WAP). After graduated from bachelor program, she began her carrier as a researcher in a local pharmaceutical company, Sanbe Farma, Bandung. Her major responsibility was to supervise researches in monoclonal antibody production and develop in-house immunohistochemistry assay which was not the common diagnostic assay used in Indonesia during that period. In 2003, she decided to pursue her education and was admitted at Biotechnology master program, Institut Teknologi Bandung, Indonesia. During her course, she constructed binary plasmid vector that carried *CB-42* gene, a candidate antigen for *C.bezziana*. At the same time she also awarded a scholarship from Japan Minister of education and culture and was admitted at Tokyo Institute of Technology, Japan. Fortunately, she could finish her master program in Indonesia in one and a half year; therefore she could start her second master program in Japan at the end of 2004. Her final project was to characterize the role of MARCH-III gene in *adipogenesis*. In 2006, she graduated from Tokyo Institute of Technology, Japan. In April 2007, she started working as a researcher in the Integrated Management for AIDS Prevention and Treatment Care (IMPACT) program. The aim of the IMPACT program (2006-2011) was to prevent, control and treat HIV among injecting drug users (IDUs) in West Java, Indonesia. This is a collaboration program between University of Padjdajaran, Indonesia and St Radboud Nijmegen University, the Netherlands. In this program, she had responsibility to improve the capability of HIV laboratory service, especially to perform HIV viral load assay as HIV treatment monitoring tool which was not available during that time. In 2009, she awarded Japan International Presidential Scholarship (JIPS) from the World Bank and began her PhD project at Dept. of Viroscience, Erasmus Medical Center, the Netherlands. During the same period she started her work as a faculty staff of School of life sciences and technology, Institut Teknologi Bandung, Indonesia (2011-present). The results of her PhD project are described in this thesis.

PhD Portfolio

Name:	Azzania Fibriani
Research group:	Department of Viroscience
Research school:	Post-Graduate Molecular Medicine
PhD period:	2009-2014
Promotors:	Prof. Dr. C.A.B. Charles Boucher
	Prof. Dr. A.J. van der Ven
Co-promotors:	Dr. Martin Schutten
	Reinout van Crevel

Education

2009-2014	PhD position at the department of Viroscience, Erasmus Medical Center, Rotterdam, the Netherlands

In-Depth courses

2009	The Indonesian-Netherlands open science meeting (KNAW)
2009	Healthy living (Molmed)
2010	The course of biology applied bioinformatics, sequences, and variations (Molmed)
2010	The basic introduction of SPSS (Molmed)
2010	The course of biological interpretation of gene expression data with ExPlain™ analysis system (Molmed)
2010	Principles of research in medicine (NIHES)
2010	Cohort studies (NIHES)
2011	English in Biomedical writing and communication (Molmed)
2013	Academic English writing course

Scientific presentations

2012	International Biological conferences, Indonesia	(poster)
2013	The 11th International congress on AIDS in Asia and the Pacific, Thailand	(poster)

International collaborations

2009-2011	Medical Faculty, Padjadjaran University, Indonesia; St Radboud Nijmegen University Medical Center, the Netherlands

Grants

2009-2014 Japan presidential scholarship, World Bank foundation

Miscellaneous

2013 Member of International AIDS society

List of publications

Hepatitis B virus prevalence, risk factors and genotype distribution in HIV infected patients from West Java, Indonesia. Fibriani A, Wisaksana R, Alisjahbana B, Indrati A, Schutten M, van Crevel R, van der Ven A, Boucher CAB. Journal of Clinical Virology, 2014, DOI: 10.1016/j.jcv.2014.01.012

Virological failure and drug resistance during first line anti-retroviral treatment in Indonesia. Fibriani A, Wisaksana R, Indrati A, Hartantri Y, van de Vijver D, Schutten M, Alisjahbana B, Sudjana P, Boucher CA, van Crevel R, van der Ven A. J Med Virol. 2013 Aug; 85(8):1394-401.

Ongoing HIV replication in cerebrospinal fluid under successful monotherapy. Bierhoff M, Boucher CA, Fibriani A, Ten Kate RW. Antivir Ther. 2013; 18(4):641-3.

Low cost HIV-1 quantitative RT-PCR assay in resource-limited settings: improvement and implementation. Fibriani A, Farah N, Kusumadewi I, Pas SD, van Crevel R, van der Ven A, Boucher CA, Schutten M. J Virol Methods. 2012 Oct; 185(1):118-23.

Response to first-line antiretroviral treatment among human immunodeficiency virus-infected patients with and without a history of injecting drug use in Indonesia. Wisaksana R, Indrati AK, Fibriani A, Rogayah E, Sudjana P, Djajakusumah TS, Sumantri R, Alisjahbana B, van der Ven A, van Crevel R. Addiction. 2010 Jun; 105(6):1055-61.

Chapter 10

Acknowledgements

Acknowledgement

Foremost, I am grateful to Allah, the owner of all mighty, for His guidance and mercy in every minute of my life. Without His guidance I will never be able to complete this PhD program.

The writing of this dissertation has been one of the most significant academic challenges I have ever had to face. Without the support, patience and guidance of the following people, this study would not have been completed. It is to them that I owe my deepest gratitude.

Prof. Charles Boucher, who undertook to act as my promoter despite his many other academic and professional commitments. Your wisdom, knowledge and commitment to the highest standards inspired and motivated me. **Dr. Martin Schutten**, my supervisor. Thank you for your support, guidance and facing me to the actual problems of HIV molecular diagnostics in Indonesia.

Prof. Andre van der Ven and **Dr. Reinout van Crevel**, my promoter and supervisor from Nijmegen, thank you for all support, knowledge and encouragements that you give to me. I never forget that you are the one who introduced me to the HIV project at Bandung. Thank you for choosing and believing me, I hope that we will still have collaboration in the future.

Thank you to **Prof. Tati Suryati Subahar Syamsudin,** Dean of School Life Science and Technology, Institut Teknologi Bandung, for all your support during my study in the Netherlands, I am looking forward for a new beginning in our beloved faculty. To **Prof. Intan Ahmad**, thank you for your encouragement, your guidance help me a lot, especially when I have to decide my present carrier to be a civil servant (*Pegawai Negri Sipil*). My sincere gratitude to my teachers and also my colleagues in Genetic and Biotechnology Molecular research group, ITB, Bapak **Sony Suhandano**, Bapak **Adi Pancoro**, Ibu **Maelita Ramdani Moeis**, Ibu **Fenny Martha Dwivany**, Ibu **Ernawati Arifin Giri-Rahman**, for all your supports and knowledge sharing all the time. It will be my honor to join this research group.

Dr. Rudi Wisasakna and **Dr. Bachti Alisjahbana,** my supervisors at Hasan Sadikin hospital, who taught me about infectious disease, specifically on HIV and its opportunistic infections. I hope our works can be a bridge between molecular biology and clinical perspectives. To Mba **Agnes Indrati**, thank you for your assistance in HIV and hepatitis laboratory works. I hope that we can extend this cooperation in the future.

Thank you to **Japan International Presidential Scholarship (JIPS)**, **World Bank,** for the grant during my study in the Netherlands. My gratitude to **Ms. Marrie Grossas** and **Ms. Karim Giggler**, for arranging the monthly stipend and everything, I hope that I can be a professional that fulfill your expectation.

For former **IMPACT** team in Bandung, **Lucas Pinxten** and **Irma Anintya**, thank you for all your support during my research. To **Inri Kusuma Dewi**, **Nadya Farah**, **Nuni**¸ and **Dwi**, what can I do without you. You are my angels who help me a lot to deal with samples collection and retrieving. I owe you a lot. May Allah reward your kindness abundantly. To **Lidya**, Mba **Rossi**, Mba **Diba**, Mba **Mawar**, mba **Nisa**, teh **Ita**, mba **Yusni**, bu **Hartiah**, kang **Irfan**, mas **Rozar**, **Mia**, **Dini**, **Yanni**, **Lika**, **Oci**, teh **Diana**, **May**, **Pepen**, **Adi**, **Shelly**, **Reynie**, mba **Erni**, and all people in IMPACT project, thank you all for the great team work. I hope that we will have a chance for extending collaboration in the future.

To **Suzan, Chantal**, **Judith**, **Anne**, **Martine**, **Jolanda**, **Jolanda (M)**, **Manon**, **Sev**, **Cedric**, **Mark, Mark (V)**, **Patricia**, **Janienne**, **Sandra**, **Thea**, **Ruud**, **Lilian**, **Hans**, **Jans**, **Robin**, **Macthold**, **Darina**, **Soeranie**, **Georgina**, **Annemiek**, **Claudia**, **Ada**, **Erhard**, **Nancy**, **David**, **Brooke**, **Marieke**, **Stalin**, **Ilona, Carola** and all my friends and colleagues in department of viroscience, thank you very much for all your support during my study in Erasmus MC. I hope that we still can meet in my defense and I do hope that we can keep in contact in the future. You will be very much welcomed if you want to visit Indonesia in the future.

To my "familiy" in the Netherlands **Aulia**, **Lia**, **Reyhan**, **Yopi**, **Dewi**, **Mufid**, **Nadira**, **Medit**, **Nisa**, **Rachman**, **Anton**, **Isna**, **Awan**, **Sekar**, **Isa**, **Gusti**, **Widi**, mba **Erma**, mas **Adi**, teh **Risah**, kang **Didin**, mba **Diyah**, pak **Deden**, teh **Irma**, pak **Satria**, **Marco**, uni **Rita**,mba **Linda**, mas **Jono**, mba **Wiwik**, **Opik**, **Dowty**, oom **Ajo**, kak **Sasa**, bang **Boy**, *keluarga besar* **PPMR**, *keluarga besar* **KMD**, and *keluaga besar* **HIMMI** , my deepest gratitude to all of you for your love and kindness that always help us to pass through our difficult time in the Netherlands.

To our beloved parents Bapak **Dachlan**, Ibu **Romlah**, Bapak **Odo**, and ibu **Entin**, for your never ending praying, thank you so much for your love, guidance, and patience, may Allah always bless you with His love and mercy. To **Ayah**, it was a hurt that I could not invite you to my master graduation ceremony in Japan, and it will be more hurt that you will not see me

completing my PhD degree in the Netherlands. Whenever I reach, I know that you will always proud of me, as I always proud of you, father. Thank you so much.

My beloved husband **Dudy** and son **Dyaz**, I am so lucky for having you two by side all the time. I am grateful for having your love, caring and patience every day. **Dyaz**, I may not always beside you all the time, but I always pray that Allah always protect and love you all the time. This is one of our goals, but our journey is still far away. May Allah always guide us in every step that we will take in the future. I will always love and proud of both of you.

Last but not the least I would like to thank to all others who have contributed to this works, *Jazzakummullahukhairan katsira*, may Allah give you abundant eternal reward.