

Correlation of Baseline Quantitative Plasma Human Immunodeficiency (HIV) Type 1 RNA Viral Load with Clinical Status and CD4+ T-cell Counts in Treatment-Naïve HIV-Positive Patients in Singapore

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Abstract

Introduction: Quantitative measurement of plasma HIV-1 RNA viral load has been available in Singapore since 30 November 1996. This study investigates the relationship, in antiretroviral-naïve, HIV-positive Singapore residents, between the baseline HIV-1 RNA viral load and clinical status at the time of quantification. The association of HIV-1 RNA viral load with CD4+ T-cell counts was also studied. **Materials and Methods:** HIV-1 RNA viral load was determined using Amplicor HIV-1 Monitor Test. One hundred and eighty subjects had baseline plasma HIV-1 RNA levels quantified during the period 30 November 1996 to 27 July 1998. They were classified into three clinical groups: A for asymptomatic infection (n = 110), B for symptomatic infection (n = 29) and C for AIDS (n = 41). **Results:** The differences between mean HIV-1 RNA levels were statistically significant (P < 0.001) for groups A and B (mean difference = -0.61 log₁₀), and for groups A and C (mean difference = -0.75 log₁₀). However, there was no statistically significant difference (P = 0.68) between groups B and C (mean difference = -0.13 log₁₀). Of those subjects with CD4+ T-cell counts measured within 30 days of viral load quantification, there were statistically significant negative correlations between HIV-1 viral load and CD4+ T-cell counts for groups A (n = 91, r = -0.536, P < 0.01) and C (n = 34, r = -0.446, P < 0.01) but not group B (n = 26, r = -0.297, P > 0.05). **Conclusion:** This suggests that the more advanced the phase of HIV infection, the higher the baseline plasma viral load and the lower the CD4+ T-lymphocyte counts.

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Key words: AIDS, HIV-1 infection, Reverse transcriptase polymerase chain reaction

Introduction

Quantitative plasma human immunodeficiency virus type 1 (HIV-1) ribonucleic acid (RNA) viral load is used clinically as a predictor of progression of HIV-1 infection to acquired immunodeficiency syndrome (AIDS) and death, and as a marker of treatment response to anti-HIV agents. Higher viral load levels have been correlated with increased risk of clinical progression of HIV disease, and that reductions in plasma virus are associated with decreased risk of clinical progression.¹⁻³ Quantitative viral load has also become a valuable tool in assessing the *in vivo* potency of treatment regimens both in clinical trial settings and in routine monitoring of HIV infection. With the use of protease inhibitors in combination therapy (also known as highly active antiretroviral therapy or HAART),

undetectable plasma viral load has become the standard treatment goal in the management of HIV infection.

Additionally in research, plasma HIV RNA quantification is used as a tool in the understanding of HIV disease pathogenesis. The capability to measure plasma viral load and the development of protease inhibitors have also contributed to the understanding of the kinetics of HIV-1 production and decay *in vivo*.^{4,5} However, this test is not used for the diagnosis of HIV infection, although it has been suggested for HIV diagnosis in infants less than 18 months old.⁶

Quantitative measurement of plasma HIV-1 RNA viral load was made available in Singapore since 30 November 1996 by the National HIV Reference Laboratory. This study investigates the relationship in antiretroviral-naïve,

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HIV-positive Singapore residents, between the first quantification of HIV-1 RNA in plasma and clinical status at the time of quantification. We also looked at the association of the first quantification of HIV-1 RNA with CD4+ T-cell counts (measured within 30 days of HIV-RNA quantification).

Materials and Methods

This is a retrospective descriptive study conducted at the Communicable Disease Centre, which is the National HIV referral centre in Singapore. The case records of patients who had plasma HIV-1 RNA quantification performed between 30 November 1996 and 27 July 1998 were systemically reviewed.

Patients

Patients less than 18 years old, those who had antiretroviral treatment prior to viral load assessment and non-residents of Singapore were excluded. Demographic data, CD4 counts measured not more than 30 days apart from viral load quantification, and clinical status at the time of viral load quantification were recorded.

The clinical status of the subjects were classified into three groups (A, B, C) depending on the clinical category they were in at the time of plasma HIV-1 RNA quantification. The three clinical categories were based on the 1993 Revised Classification System for HIV infection for adolescents and adults.⁷

Clinical category A constituted persons with acute (primary) HIV illness or with asymptomatic HIV infection with or without persistent generalised lymphadenopathy. Category B consists of HIV infected patients with symptomatic conditions that are not listed in category C and that meet at least one of the following criteria:

- 1) the conditions are attributed to HIV infection or are indicative of a defect in cell-mediated immunity; or
- 2) the conditions are considered by physicians to have a clinical course or to require management that is complicated by HIV infection.

Examples of some conditions are oropharyngeal candidiasis, oral hairy leukoplakia, and herpes zoster. Patients in clinical category C had at least one of the conditions listed in the AIDS surveillance case definition. In brief, Group A comprised patients with asymptomatic HIV infection, Group B with symptomatic HIV infection and Group C with AIDS.

Laboratory Analyses

The National HIV Reference Laboratory uses reverse transcriptase polymerase chain reaction (RT-PCR) method to measure viral load, specifically Amplicor HIV-1 Monitor Test by Roche Diagnostic Systems. This test comprises five major processes:⁸

- 1) specimen preparation,
- 2) reverse transcription of target RNA to generate cDNA,
- 3) PCR amplification of target cDNA using HIV-1 specific complementary primers,
- 4) hybridisation of the amplified products to oligonucleotide probes, and
- 5) the detection of the probe-bound amplified products by colorimetric determination.

From November 1996 to January 1997, Amplicor HIV-1 Monitor Test version 1.0 was used and from February 1997 to December 1997, version 1.0+ was used. Version 1.0+ assay contains an additional two PCR primers and this has significantly improved the efficiency of quantification for subtypes A and E.⁹ Hence, for all HIV-1 subtype E infected subjects in this study, whose samples were tested using version 1.0 assay, repeated testing was done using version 1.0+ assay. From January 1998 onwards, Amplicor HIV-1 Monitor Test version 1.5 was used. With the utilisation of a new primer pair and a new thermal cycling profile, version 1.5 amplifies HIV-1 subtypes A through G with equal efficiency.^{10,11} Standard specimen processing was done for all samples.

CD4+ T-cell counts were determined using flow cytometry.

Statistical Analyses

Amplicor HIV-1 Monitor Test with standard specimen processing gives valid results within the linear range of 400 to 750,000 copies per mL. Viral load levels that were below the lower limit of 400 copies per mL and beyond the upper limit of 750,000 copies per mL were assumed to be valid for the purpose of statistical analyses for this paper. Statistical analyses were done using Microsoft Excel 97 and SPSS 8.0 for Windows. All statistical tests were conducted at 5% level of significance.

Results

A total of 328 patients with first-time plasma HIV RNA-1 quantification done between 30 November 96 and 27 July 98 were reviewed. Of those excluded, 37 were non-residents of Singapore, 3 were younger than 18 years and 108 had already commenced antiretroviral treatment. Out of 180 persons who met our inclusion criteria, 110 were in group A, 29 in group B and 41 in group C. The demographics of the patients are shown in Table I.

Baseline HIV-1 RNA Levels

The respective mean plasma HIV-1 RNA levels in these three groups A, B and C were 162,093 (5.21 log₁₀) copies/mL (range 109 to 2,148,837 copies/mL), 370,795 (5.57 log₁₀) copies/mL (range 24,894 to 1,620,076 copies/mL) and 636,541 (5.80 log₁₀) copies/mL (range 9,512 to 2,924,292 copies/mL) (Fig. 1).

TABLE I: DEMOGRAPHICS OF STUDY POPULATION

	Group A Asymptomatic	Group B Symptomatic	Group C AIDS	Total
No. of patients	110	29	41	180
Age (y)				
Average	36	41	41	38
Range	20-63	23-76	26-77	20-77
Sex				
Male	97	28	38	163
Female	13	1	3	17
Ethnic group				
Chinese	95	24	34	153
Indian	7	2	2	11
Malay	5	1	2	8
Others	3	2	3	8
Time (in months) between date of diagnosis and viral load quantification	18.3 (0.3-120.1)	6.9 (0.0-57.3)	7.0 (0.0-89.5)	13.9 (0.0-120.1)
No. with available CD4 counts	91	26	34	151

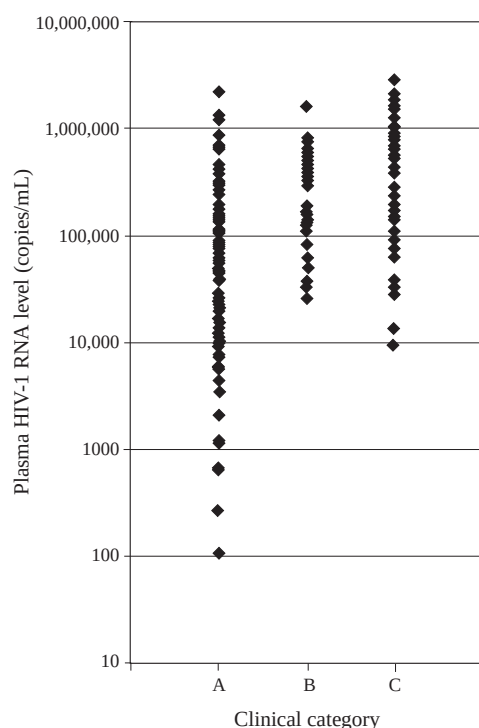


Fig. 1. Distribution of HIV-1 RNA level by clinical category.

In group A, 1 individual had a viral load that was below the lower limit of the linear range of the Amplicor HIV-1 Monitor Test, i.e. below 400 copies/mL. There were 4, 3 and 14 persons in group A, B and C respectively who had

viral loads above the linear range of the Amplicor HIV-1 Monitor Test, i.e. above 750,000 copies/mL.

Due to the expected right skew in distribution of the HIV-1 RNA levels, the base 10 log value of the number of copies/mL was used in the statistical analysis. The base 10 log values were found to be normally distributed by the one-sample Kolmogorov-Smirnov test ($P=0.32$). Variances within the three groups were tested equal using Levene statistics. By achieving normality the usual parametric tests could be applied.

Pairwise comparisons between the three groups were done using Tukey HSD and Scheffe statistics. Both showed that the differences between mean HIV-1 RNA levels were statistically significant ($P < 0.001$) for groups A and B (mean difference = $-0.61 \log_{10}$; 95% confidence interval using Tukey HSD = $-0.95 \log_{10}$ to $-0.28 \log_{10}$), and for groups A and C (mean difference = $-0.75 \log_{10}$; 95% confidence interval using Tukey HSD = $-1.05 \log_{10}$ to $-0.46 \log_{10}$). However, there was no statistically significant difference ($P = 0.68$ by Tukey HSD and $P = 0.71$ by Scheffe) in mean plasma HIV-1 RNA levels for group B and C (mean difference = $-0.13 \log_{10}$; 95% confidence interval using Tukey HSD = $-0.52 \log_{10}$ to $0.25 \log_{10}$).

CD4+ T-cell Counts Versus HIV-1 RNA Levels

In groups A, B and C, 91, 26 and 34 patients respectively had CD4+ T-cell counts measured not more than 30 days apart from HIV-1 RNA quantification. Figures 2 to 4 show the graphical representation of these values for the three

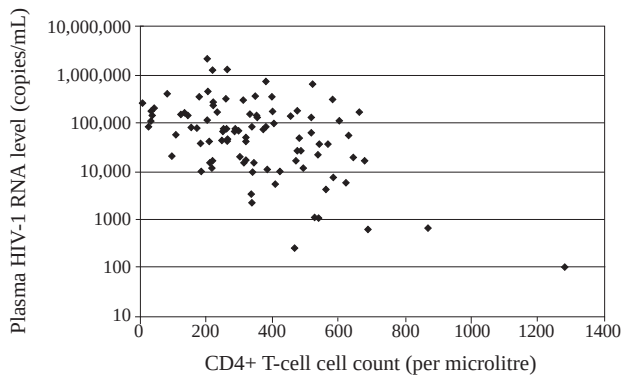


Fig. 2. HIV-1 RNA and CD4+ levels for Group A.

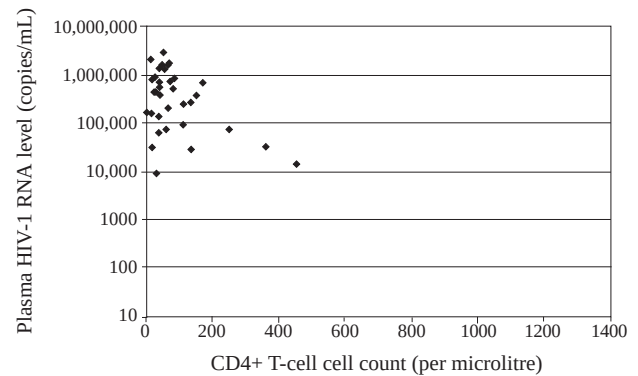


Fig. 4. HIV-1 RNA and CD4+ levels for Group C.

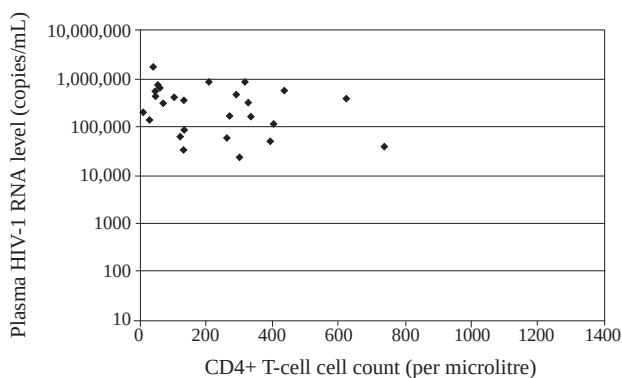


Fig. 3. HIV-1 RNA and CD4+ levels for Group B.

groups. The average CD4+ T-cell counts for groups A, B and C were $354 \times 10^6/L$ (range 11 to $1,285 \times 10^6/L$), $225 \times 10^6/L$ (range 11 to $733 \times 10^6/L$) and $84 \times 10^6/L$ (range 1 to $453 \times 10^6/L$), respectively.

The Pearson's correlation coefficient was calculated to assess the correlation of CD4+ T-cell counts with plasma HIV-1 RNA levels. Overall, there was a statistically significant negative correlation ($r = -0.593$, $P < 0.01$, 95% confidence interval = -0.68 to -0.47) between plasma HIV-1 RNA levels (base 10 log values) and CD4+ T-cell counts. When each group was tested separately, there were statistically significant negative correlations for groups A ($r = -0.536$, $P < 0.01$, 95% confidence interval = -0.66 to -0.37 ; Fig. 2) and C ($r = -0.446$, $P < 0.01$, 95% confidence interval = -0.68 to -0.12 ; Fig. 4) but not group B ($r = -0.297$, $P > 0.05$, 95% confidence interval = -0.61 to 0.10 ; Fig. 3).

Discussion

Plasma HIV-1 RNA can be detected at various stages of HIV infection. In the first 3 to 6 weeks of exposure to HIV, infected individuals generally develop a brief, acute mononucleosis-like syndrome. This period is associated with high levels of plasma viraemia.^{12,13} This is followed by

the development of detectable humoral and cellular immune response to HIV virus with a corresponding dramatic decline of plasma viraemia. After seroconversion, infected individuals typically enter a clinically stable, asymptomatic period that can last for years.¹⁴ During this asymptomatic or steady state of HIV infection, the high rates of virus production and infection of CD4+ cells are balanced by equally high rates of virus clearance, death of infected CD4+ cells and replenishment of CD4+ cells. This dynamic process results in relatively stable levels of both plasma HIV-1 RNA and CD4+ cells.^{4,5} The rate of disease progression correlates to the level of HIV-1 RNA at the steady state, i.e. the higher the plasma HIV-1 RNA level, the faster the disease progression. As the disease progresses, there is a gradual depletion of CD4+ T lymphocytes. At the advanced phase of HIV infection, there is a rapid rise in plasma viraemia, leading to rapid destruction of CD4 cells with resultant severe immunodeficiency, multiple opportunistic infections, malignancies and death.¹⁵

While the National HIV Reference Laboratory uses RT-PCR method to measure viral load, other commercially available assay methods are nucleic acid sequence-based amplification (NASBA) [NucliSens HIV-1 QT by Organon Teknika] and signal amplification by branched DNA (bDNA) technique [Quantiplex HIV-1 RNA Assay by Chiron Diagnostics]. Concordance varies between these three different assays,¹⁶⁻¹⁸ so it is usually recommended that the same test be used for individual patient follow-up. A concordant result being defined as a difference of less than $0.5 \log_{10}$ copies/mL between assays.

When the National HIV Reference Laboratory first started quantifying plasma HIV-1 RNA levels, the only choice available then was Amplicor HIV-1 Monitor Test version 1.0. Only after a couple of months was Quantiplex HIV-1 RNA Assay version 1.0 made available. The Laboratory compared both assays and found that Amplicor HIV-1 Monitor Test version 1.0 was consistently more sensitive than Quantiplex version 1.0 in detecting subtype B samples,

but not so for subtype E samples. The Laboratory later switched to Amplicor HIV-1 Monitor Test version 1.0+ which gave better results to the subtype E virus, without compromising the sensitivity in detecting subtype B virus. This is an important consideration as the majority of HIV patients in Singapore are infected with either HIV-1 subtype B or E virus.^{19,20} The NASBA assay was not compared because the Laboratory was not able to access the test kit until recently. Ultrasensitive specimen processing with Amplicor HIV-1 Monitor Test was started with version 1.0+ from June 1997, and with version 1.5 from January 1998 onwards. Ultrasensitive specimen processing is not routinely used clinically for baseline assessment but for monitoring of treatment response as it gives valid results within a lower linear range of 50 to 75,000 copies per mL.

The findings of this study concur that HIV-1 RNA levels were higher at advanced state of HIV infection as seen in the significant difference in mean plasma HIV-1 RNA levels for our asymptomatic (group A) patients compared to our symptomatic (group B) and our AIDS (group C) patients. The mean plasma HIV-1 RNA level increased with progression of clinical status. However, plasma HIV-1 RNA levels could not predict the clinical category patients were in.

HIV-1 RNA levels indicate the magnitude of HIV replication, and CD4+ lymphocyte count indicates the extent of HIV-induced immune damage. Hence, we would expect a negative correlation between these two markers as the disease progresses. This is reflected in our asymptomatic (group A) and AIDS (group C) patients.

Only 1 patient in this study had a CD4+ T-cell count above $1000 \times 10^6/L$ ($1285 \times 10^6/L$) and his corresponding HIV-1 RNA level was 109 copies/mL. Plasma HIV-1 RNA level was done in May 1997 about 5½ months after diagnosis. By May 1999, he had remained asymptomatic, his plasma HIV-1 RNA level had risen to 1128 copies/mL and CD4 level had dropped slightly to $1014 \times 10^6/L$. For this patient, we could not accurately pinpoint the time when he was infected, but in view of his low baseline viral load and normal immune function over 2 years of follow-up, he may remain asymptomatic for a very long period, i.e. known as an HIV slow progressor or non-progressor.

Recommendations for treatment of HIV infection using clinical status, CD4+ T-cell counts and plasma HIV-1 RNA levels have been made by the Panel on Clinical Practices for Treatment of HIV Infection (convened by the US Department of Health and Human Services).²¹ Briefly, any symptomatic or AIDS patients should receive antiretroviral therapy. Also, any asymptomatic patient with either CD4+ T-cell count of less than $500 \times 10^6/L$ or plasma HIV-1 RNA level by RT-PCR of more than 20,000 copies/mL should be offered antiretroviral therapy. A

quick analysis of the 110 asymptomatic patients in our study revealed 80 subjects (72.7%) with more than 20,000 copies/mL. Out of the remaining 30 subjects, 17 had CD4+ T-cell counts of less than $500 \times 10^6/L$. This would mean that under current US Department of Health and Human Services recommendations, 97 (88.2%) asymptomatic subjects would require antiretroviral therapy. This would be in addition to our symptomatic and AIDS patient population. With this large percentage requiring treatment, there will be healthcare cost implications as triple therapy is expensive.

The baseline plasma HIV-1 RNA levels in this cohort of local HIV-positive population indicate concordance with work done in western studies.²² Higher baseline plasma viral load and lower CD4+ T-lymphocyte counts were noted in more advanced phase of HIV infection. From this baseline study, it provides us the opportunity to assess the effects of antiretroviral treatment, in particular, the protease inhibitors, on HIV-1 RNA levels for patients managed at our centre.

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