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Pooled nucleic acid testing to identify antiretroviral treatment failure during HIV infection in Seoul, South Korea

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Abstract

Background—There have been various efforts to identify less costly but still accurate methods for monitoring the response to HIV treatment. We evaluated a pooling method to determine if this could improve screening efficiency and reduce costs while maintaining accuracy in Seoul, South Korea.

Methods—We conducted the first prospective study of pooled nucleic acid testing (NAT) using a 5 minipool + algorithm strategy versus individual viral load testing for patients receiving antiretroviral therapy (ART) between November 2011 and August 2012 at an urban hospital in Seoul, South Korea. The viral load assay used has a lower level of detection of 20 HIV RNA copies/ml, and the cost per assay is US\$136. The 5 minipool + algorithm strategy was applied and 43 pooled samples were evaluated. The relative efficiency and accuracy of the pooled NAT were compared with those of individual testing.

Results—Using the individual viral load assay, 15 of 215 (7%) plasma samples had more than 200 HIV RNA copies/ml. The pooled NAT using the 5 minipool + algorithm strategy was applied to 43 pooled samples; 111 tests were needed to test all samples when virologic failure was defined at HIV RNA > 200 copies/ml. Therefore, 104 tests were saved over individual testing, with a relative efficiency of 0.48. When evaluating costs, a total of US\$ 14,144 was saved for 215 individual samples during 10 months. The negative predictive value was 99.5% for all samples with HIV RNA > 200 copies/ml.

Conclusions—The pooled NAT with 5 minipool + algorithm strategy seems to be a very promising approach to effectively monitor patients receiving ART and to save resources.

Keywords

HIV; pooling; antiretroviral therapy; viral load; virologic failure

Introduction

Routine viral load monitoring to detect the failure of antiretroviral therapy (ART) in suppressing HIV replication is an important clinical practice when resources are available [1]. Monitoring for virologic failure during ART is important to limit the development and transmission of HIV drug resistance [2–4]. The current clinical recommendation for the monitoring of viral loads during ART is every 3–6 months, and this monitoring strategy has proven cost-effective in settings with sufficient resources [5]. However, because of restrictions in capabilities and costs, the use of viral loads to monitor virologic failure during ART is not recommended or performed in most resource-limited settings [6–8]. In efforts to save resources, some have proposed detailed historical, hematological, immunological, and clinical monitoring instead [7,9,10].

Previous studies have demonstrated that measures that do not investigate active viral replication are not sufficient to detect virologic failure and foster the spread of drug resistance within a population [6,10,11]. Less expensive methods to monitor virologic failure during ART are thus required in resource-limited settings to make virologic monitoring feasible. Various efforts have demonstrated that screening for HIV RNA among people presenting for HIV testing or blood donation can be used efficiently to identify individuals who are acutely infected with HIV despite a negative HIV antibody test [12–14]. Because testing for HIV RNA in each blood sample would be expensive, a strategy to pool blood samples from a group of individuals and perform one HIV RNA assay on a pooled sample is commonly used [12–14]. If the pool tests positive, individual samples or pools of smaller size might be tested again to identify affected individuals. This strategy has been found to be a highly efficient and affordable means of identifying individuals with acute HIV infection [13].

Recently, investigators have applied pooling algorithms to screen for virologic failure during ART for retrospective cohorts of patients in the USA, Mexico, and South Africa [15–17]. These studies found that pooling algorithms could improve efficiency and reduce the costs of virologic monitoring while maintaining accuracy. However, to further generalize the usefulness of the pooling strategy for identifying virologic failure, the strategy should be further validated prospectively within diverse regions and populations, since the characteristics of the strategies such as relative efficacy, accuracy, and cost-effectiveness can be affected by regional HIV clinical practices and characteristics of the HIV population.

South Korea has a relatively low HIV prevalence of less than 0.1% [18]. A total of 8544 cumulative HIV infections were identified and 7032 persons were still alive in South Korea as of 2011, and the number of newly diagnosed individuals is increasing annually. According to the World Bank criteria, South Korea is a high-income country [19]. Viral load testing is a routine clinical practice in HIV care during ART at Korean medical institutes, and it costs US\$ 136 per test, which includes the cost of the technician performing the assay, as well as the cost of the assay itself. With 4000 individuals receiving ART in South Korea, if viral loads were used every 3 months to monitor these patients, then virologic monitoring in South Korea would cost US\$ 2,176,000 annually. Although high-income countries such as South Korea may be able to afford the budget for individual viral load testing, having a less expensive option for virologic monitoring could have a considerable impact on the overall HIV care budget. To apply less expensive methods for virologic monitoring in resource-limited countries, a feasible method should be further validated with comparison to standard methods in a prospective way. Therefore, we prospectively evaluated if a pooling method could improve efficiency and reduce costs while maintaining accuracy in Seoul, South Korea.

Methods

Study population

This study was approved by the institutional review board (No. 4-2011-0477) and was conducted at a tertiary care teaching hospital in Seoul, South Korea. Informed consent was obtained from the subjects. We consecutively enrolled HIV-infected patients who had received their ART regimen, containing at least three agents, between November 2011 and August 2012.

Nucleic acid testing

All nucleic acid testing (NAT) was performed using the Cobas AmpliPrep/Cobas TaqMan HIV-1 Test, version 2.0 (Roche Molecular Systems, Pleasanton, CA, USA) in accordance with the manufacturer's instructions. The NAT used has a lower level of detection of 20 HIV RNA copies/ml, and the cost per assay in the institute is US\$ 136. Based on previous studies [15,16,20], we compared two NAT screening methods to evaluate the identification of virologic failure during ART: testing samples individually and with a 5 minipool + algorithm method. Samples were individually tested and placed within the 5 minipool + algorithm platforms based on consecutive orders. Individual sample testing and reformatting into minipools were performed by a single technician who was blinded to the clinical information of the individuals.

The 5 minipool + algorithm method was conducted as described previously [15,20]. These methods were based on Dorfman's [21] two-stage 'minipool' approach, where a fixed number of samples is incorporated into one pool. If the pooled samples produced a viral load value above the lower limit of detection for the pool, then individual samples would be confirmed successively and subtracted from the pooled sample quotation until the lower limit of detection for the pool was fulfilled [15,20]. Algorithm thresholds for virologic failure of an individual sample were defined a priori as 200, 500, 1000, and 1500 HIV RNA copies per milliliter, translating to thresholds of 40, 100, 200, and 300 HIV RNA copies per milliliter to define a positive pool. These thresholds were chosen based on the dilutional effect of combining 5 individual samples in a pool. A search and test algorithm implemented in a web-based program (<http://mepac.ucsd.edu>) that incorporates individual and pooled viral load values into the resolution of positive pools was used, as described previously [15,16,20].

Efficiency, accuracy, and cost savings

Test characteristics, including relative efficiency compared with individual testing and accuracy, were determined for the 5 minipool + algorithm platform at each testing threshold. Relative efficiency was defined as the percentage of assays saved by pooling methods compared with individual testing. Accuracy was calculated as the negative predictive value, and each algorithm threshold was evaluated for its ability to detect virologic failure at levels of 200, 500, 1000, and 1500 HIV RNA copies per milliliter. Cost savings were estimated based on the relative efficiency of the 5 minipool + algorithm platform at each threshold.

Results

Patient characteristics

Between November 2011 and August 2012, 150 patients receiving ART were evaluated. Table I shows the baseline characteristics of the patients. Using the individual viral load assay, 48 of 215 (22.3%) plasma samples contained more than 20 HIV RNA copies/ml and 15 of 215 (7%) plasma samples had more than 200 HIV RNA copies/ml.

Relative efficiency and accuracy

The pooled NAT using the 5 minipool + algorithm strategy was applied to 43 minipools of 5 samples, representing 215 individuals. One hundred and eleven tests were performed to complete testing of all samples when virologic failure was defined as HIV RNA ≥ 200 copies/ml. Compared to individual testing, 104 tests were saved and the relative efficiency was 0.48. Converting the tests to cost, a total of US\$ 14,144 was saved for 215 individual samples during 10 months. The negative predictive value was 99.5% for the cut-off point of HIV RNA ≥ 200 copies/ml. The pooled NAT was unable to identify one sample with a viral load of 213 copies/ml.

When virologic failure was defined as 500 HIV RNA copies/ml, a total of 98 viral load tests were performed, resulting in a relative efficiency of 0.54 and cost savings of US\$ 15,912 compared to individual testing. When virologic failure was defined as 1000 HIV RNA copies/ml, a total of 89 viral load tests were performed, resulting in a relative efficiency of 0.59 and cost savings of US\$ 17,136 compared to individual testing. When virologic failure was defined as 1500 HIV RNA copies/ml, a total of 89 viral load tests were performed, resulting in a relative efficiency of 0.59 and cost savings of US\$ 17,136 compared to individual testing. All negative predictive values were 100% for cut-off points of HIV RNA 500, 1000, and 1500 copies/ml.

Discussion

There have been various endeavors worldwide to find a less costly but still accurate method for monitoring the response to treatment. One such endeavor is the pooled testing of samples. Pooled HIV RNA testing is the performance of HIV RNA testing of pools made from multiple patient specimens, which is efficient in diagnosing acute HIV infection [13,22]. The use of NAT on pooled blood samples to identify virologic failure is not the same as using NAT on pooled blood samples to identify instances of acute HIV infection. First, during acute HIV infection the level of HIV RNA is much higher than when ART fails to fully suppress HIV replication [14,23,24]. Second, among people tested for acute HIV or donating blood, the prevalence of acute HIV infection is rare, while for patients receiving ART the prevalence of virologic failure would be much greater and, therefore, require more resolution testing of pooled samples than would be required for acute HIV infection screening. Third, the variability of the viral load assay may be more problematic and cause instances of virologic failure of ART to be missed at the lower levels of viral replication observed during ART failure. Fourth, to maximize the cost efficacy and overall clinical usefulness of the strategy, the number of pooled and individual NAT assays, time from pooled screening to individual viral load results, and sources of error, such as specimen contamination and assay variability, must be minimized. However, pooled testing using a clinically appropriate viral load threshold has been shown to be an accurate and efficient method for detecting virologic failure in patients receiving ART in retrospective cohorts [15,17,20]. This study was prospectively planned to evaluate if a pooling method could improve efficiency and reduce costs while maintaining accuracy in Seoul, South Korea.

In our study, the 5 minipool + algorithm method was compared with individual viral load sampling. The study showed several significant results: (1) in terms of cut-off points of HIV RNA 200, 500, 1000, and 1500 copies/ml, the relative efficiency was 0.48, 0.54, 0.59, and 0.59, respectively; (2) a total of US\$ 14,144, US\$ 15,912, US\$ 17,136, and US\$ 17,136 for cut-off points of HIV RNA 200, 500, 1000, and 1500 copies/ml, respectively, was saved for 215 individual samples during 10 months; and (3) negative predictive values were more than 99.5% for all cutoff points. Some authors have reported that the prevalence of virologic failure in society is associated with relative efficiency [15,16,20]. For example, with a prevalence of virologic failure of 22%, the relative efficiency of the 5 minipool + algorithm

method was around 0.3 [20], and with a prevalence of virologic failure of 9%, relative efficiency was 0.5 [20]. This likely lowered the pooling efficiency by increasing the overall prevalence of detectable viremia and the need for individual retesting, and indeed the relative efficiencies observed were lower [16]. The prevalence of virologic failure in our population was a priori estimated to be 9%, but it was 7% for viral loads > 200 copies/ml. Our higher relative efficiency than in other studies is likely to be related to the low prevalence of virologic failure in our population.

Overall, pooled viral load testing results in a decrease in sensitivity due to the dilutional effect of combining multiple individual samples and can increase the turnaround time of results [20]. Since the turnaround time can impact the clinical management of patients, pooled algorithm methods are likely best implemented in laboratories with a high throughput of samples. In our study, the calculated time for one technician to perform 43 viral load assays on 215 individual samples was not evaluated; however in similar research, the turnaround time was estimated to be approximately 5–7 days [15]. This factor will be evaluated in future studies. To further evaluate the usefulness of the pooled NAT, additional costs for storing and retrieving samples from freezers for repeated testing should be considered, and the risk of operator errors should be minimized to increase the usefulness of the strategy. Despite these limitations, our study shows that pooled testing combined with algorithms that incorporate the quantitative results of the viral load assay has excellent accuracy. Similar to previous reports from across the world, the pooled NAT was found to decrease the costs of virologic monitoring of Korean patients on ART.

In conclusion, the pooled NAT with the 5 minipool + algorithm strategy seems to be a very promising approach to effectively monitor patients receiving ART and to save resources. This could be a feasible and cost-saving method to monitor for virologic failure during ART. Further investigations on the turnaround time are needed for optimal clinical evaluation.

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Table I

Baseline demographic characteristics of the patients.

Characteristics	Values
Age, y, median (range)	48 (21–80)
Sex, <i>n</i> patients (%)	
Male	137 (91%)
Female	13 (9%)
HAART duration, days	1898 (56–6830)
HAART regimen, <i>n</i> patients (%)	
PI based	88 (59%)
NNRTI based	48 (32%)
Integrase inhibitor based	7 (5%)
Other	7 (5%)
CD4 cell count at the time of cells/mm ³ , median (range)	486 (32–1252)
Viral loads at the time of sampling, <i>n</i> samples (%)	
< 20 copies/ml	167 (77.7%)
20–199 copies/ml	33 (15.3%)
200–499 copies/ml	3 (1.4%)
500–1499 copies/ml	2 (0.9%)
1500 copies/ml	10 (4.7%)

HAART, highly active antiretroviral therapy; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor.

Table II

Test characteristics of the 5 minipool + algorithm platform compared with individual viral load testing.

Algorithm thresholds (HIV RNA copies/ml)	Assays for samples (<i>n</i> = 215)	Relative efficiency	NPV (%)	Saved cost per sample (US\$) ^a
Individual testing				
NA	215	0	100	0
5 minipool + algorithm platform				
200	111	0.48	99.5	14,144
500	98	0.54	100	15,912
1000	89	0.59	100	17,136
1500	89	0.59	100	17,136

NPV, negative predictive value; NA, not applicable.

^aCosts are in US dollars.