

Virologic Diagnosis and Follow-up of Children Born to Mothers Infected by HIV-1 Group O

To the Editor:

HIV-1 is subdivided into groups M, N, and O. Group O was first identified in 1994.^{1,2} Diagnosis and follow-up of infants born to mothers infected by HIV-1 group O have been problematic, owing to a lack of methods adapted to these variants. Recently, viral RNA quantification in plasma was made possible by the advent of the LCx HIV RNA QT kit (Abbott, Chicago, IL) and our own real-time polymerase chain reaction (PCR) method based on Lightcycler (LC) technology (Roche Diagnostics GmbH, Germany).³ The lack of a commercial screening kit for HIV-1 group O proviral DNA means that infection cannot be confirmed or ruled out in children with undetectable plasma viral RNA, which is notably the case during antiretroviral prophylaxis. To improve the diagnosis and management of vertical HIV-1 group O transmission, we have developed a real-time PCR method on LC that specifically detects the proviral DNA of these variants and have also optimized our quantitative plasma RNA assay method. We used these 2 methods to diagnose and monitor 4 children born in France to mothers infected by HIV-1 group O.

METHODS AND PATIENTS

Peripheral blood mononuclear cells (PBMCs) and plasma were separated by Ficoll-Hypaque density gradient centrifugation and stored at -80°C .⁴ DNA was extracted with the QIAamp DNA Blood Mini Kit (Qiagen GmbH, Germany) as recommended by the manufacturer. The final elution volume was 50 μL . The amount of DNA in each extract

was determined by real-time PCR specific for the albumin gene,⁵ with human genomic DNA as standard (Roche Diagnostics). RNA was extracted using the Magna Pure LC device and the Total Nucleic Acid kit, according to the manufacturer's instructions (Roche Diagnostics). The initial plasma volume was 200 μL and volume of eluate was 50 μL . PCR detection of proviral DNA and plasma RNA quantification by RT-PCR were based on the same primers P1 5'-CTCAATAAAGCTTGCCCTTGA-3' and P2 5'-CGCCACTGCTAGAGATTTT-3' and the probe FAM-5'-AAGCAGTGTGTGCTCATCTGTTG-3'-Eclipse Dark Quencher (Eurogentec, Seraing, Belgium). These primers amplify a fragment of 111 bp located in the long terminal repeat region of HIV-1 group M and O strains. Revelation is based on a group O-specific Double-Dye probe as previously published,³ but to reduce background during revelation, the TAMRA quencher has been replaced by the Eclipse Dark quencher.

Proviral DNA was PCR-amplified using the LC-Faststart DNA master hybridization probes kit. The LC master mix (2 μL) was mixed with 4 mM MgCl_2 , 0.5 μM each primer, and 0.5 μM probe. A variable volume of extract, containing 500 ng of DNA, was added, and the final volume was made up to 20 μL with water. Amplification was carried out as follows: 95°C for 10 minutes (1 cycle), denaturation at 95°C for 10 seconds, and annealing at 58°C for 30 seconds (45 cycles). A synthetic plasmid³ diluted with human DNA to 2 concentrations (5000 and 50 copies/500 ng of DNA) served as the positive control for each run. To determine the sensitivity of the PCR method for proviral DNA, the plasmid standard was diluted in human DNA to 10, 5, and 1 copies/500 ng of DNA, and each concentration was tested 5 times.

RT-PCR previously described in a 2-step procedure was simplified.³ One-step RT-PCR was performed with the LC-RNA Master Hybridization Probes kit (Roche Diagnostics) with 2.5 mM

$\text{Mn}(\text{OAc})_2$, 0.5 μM each primer, and 0.5 μM probe. Ten microliters of RNA solution was added to 10 μL of this mixture. Amplification was carried out as follows: reverse transcription 61°C for 30 minutes, 95°C for 30 seconds (1 cycle), denaturation 95°C for 1 second, annealing 58°C for 5 seconds, elongation 65°C for 30 seconds, and reading 55°C for 3 seconds (45 cycles). The quantification standard was the supernatant of an HIV-1 group O clade A strain (YBF32) amplified by cell culture and quantified 4 times by LCx. The sensitivity of this new version of the assay was determined by testing samples containing 500, 200, and 100 copies/mL, 5 times each.

We then tested clinical samples from the 4 infants (babies 1, 2, 3, and 4) and their mothers (mothers 1, 2, 3, and 4). The 4 mothers, all from Cameroon, were diagnosed with HIV-1 group O infection by means of specific peptide-based assays, as previously described, with confirmation by sequencing.^{6,7} The mothers and babies were screened for proviral DNA just after delivery, and the infants were retested later to confirm the initial results. Plasma RNA was quantified in the same samples, in parallel, by means of optimized real-time RT-PCR, and by using the LCx HIV RNA QT kit according to the manufacturer's procedures.

RESULTS

In the proviral DNA screening test, all the standards containing 10 and 5 copies/500 ng of DNA were positive, but only 40% of the samples containing 1 copy/500 ng of DNA were positive. The detection limit of the method was thus set at 5 copies/500 ng of DNA. The sensitivity of the plasma RNA quantification assay was reevaluated: all the standards containing 500 and 200 copies/mL were positive, but only 60% of standards containing 100 copies/mL were positive. The quantification limit was thus set at 200 copies/mL.

Proviral DNA was detected in all the mothers, confirming that the PCR

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TABLE 1. Follow-up of Mothers and Babies

Patients	Proviral DNA PCR	1 st Viral Load (log)				2 nd Viral Load (log)				3 rd Viral Load (log)			
		Day*	Treatment	LCx	LC	Day	Treatment	LCx	LC	Day	Treatment	LCx	LC
Mother 1	+	D4	AZT + 3TC + LPV/r	<2.2†	<2.3	D49	AZT + 3TC + LPV/r	<2.2	<2.3	ND	ND	ND	ND
Baby 1	-	D4	AZT	<2.2	<2.3	D41	AZT	<2.2	<2.3	D132	None	<2.2	IQ
Mother 2	+	D(-)3	AZT	3.4	3.2	D123	None	3.4	3.7	D214	None	3.3	2.9
Baby 2	-	D1	AZT	<2.2	<2.3	D39	AZT	<2.2	IQ	D81	None	<2.2	<2.3
Mother 3	+	D(-)8	AZT + 3TC	2.4	2.0‡	D127	None	IQ	4.3	ND	ND	ND	ND
Baby 3	-	D6	AZT	<2.2	<2.3	D43	AZT	IQ	<2.3	D85	None	IQ	<2.3
Mother 4	+	D20	None	4.4	4.6	D109	AZT + 3TC + TDF	<1.7§	<2.3	D165	AZT + 3TC + TDF	<1.7	<2.3
Baby 4	+	D89	None	4.9	IQ	D152	None	5.6	5.7	D186	None	3.5	3.1

*D = Day after the childbirth, D(-) = day before the childbirth.

†Input volume 200 µL.

‡Nonlinear result.

§Input volume 1 mL.

AZT, zidovudine; 3TC, lamivudine; LPV/r, lopinavir/ritonavir; TDF, tenofovir; ND, Not determined; IQ, insufficient quantity.

method amplified transmissible virions (Table 1). Babies 1, 2, and 3 were negative for proviral DNA, while baby 4 was positive. Good agreement on plasma viral load values was obtained with the LCx and LC real-time PCR methods (Table 1). Viral load was below the quantification cutoff of both techniques in mother 1, and was quantifiable in mothers 2 and 3, with a difference of <0.5 log between the 2 methods. Viral load in mother 4 was 4.4 log in the LCx technique and 4.6 log in the LC technique. Viral load assay confirmed the negativity of babies 1, 2, and 3, and confirmed that baby 4 was infected. PBMCs from baby 4 were then co-cultured with phytohemagglutinin-activated PBMCs from seronegative subjects. P24 antigen was detected in the culture supernatant, and proviral DNA was detected in end-cultured cells with our PCR method.

DISCUSSION

Our PCR developed for group O proviral DNA can be used to diagnose the infection in infants who receive antiretroviral treatment from birth and who have undetectable viral load. Moreover, we have optimized and simplified the plasma RNA assay by including automated extraction, a one-step RT-PCR, and a new quencher. The standard range is now prepared from a culture superna-

tant, which is treated identically to clinical samples. The quantification limit is 200 copies/mL, representing a marked improvement over the previous assay (1500 copies/mL).³

To date, 65 HIV-1 group O-infected patients resident in France have been identified, two-thirds of whom are women of child-bearing potential, and mother-to-child transmission of HIV-1 group O has been documented.⁸ The precise risk of transmission is not known, owing to the small number of patients.

In our study, none of the 3 children whose mothers were diagnosed sufficiently early in pregnancy and were treated at the end of pregnancy was infected. The serologic status of mother 4, whose baby was infected, was not known at the time of delivery, and she was therefore not treated. Her CD4 cell count was 78/mm³, pointing to high viral load. Antiretroviral treatment started after delivery drove her viral load below the detection limit (Table 1). At age 6 months, the child developed rapidly progressive disease with early signs of HIV encephalopathy, profound immune deficiency (CD4 cell count 400/mm³), and pulmonary interstitial lymphoid disease. Treatment with the zidovudine, lamivudine, and lopinavir/ritonavir combination was

started on day 186 of life and drove viral load below the detection limit (data not shown).

In conclusion, it is now possible to diagnose and monitor HIV-1 group O infection with specific serologic and molecular tools. Proviral DNA screening can be used to diagnose children born to infected mothers, and also patients with undetectable plasma viral RNA, as recently reported with LCx and LC technology.^{3,9}

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The Influence of Hepatitis C Virus Coinfection on the Risk of Lipid Abnormalities in a Cohort of HIV-1-Infected Patients After Initiation of Highly Active Antiretroviral Therapy

To the Editor:

Highly active antiretroviral therapy (HAART) has significantly reduced

AIDS-related morbidity and mortality.¹ The improved survival made possible by HAART has brought to light the potential long-term complications of drug therapy, including the risk of cardiovascular disease.² Several metabolic disorders such as dyslipidemia, diabetes, and alteration of body fat composition^{2–4} can develop or worsen in patients during HIV therapy. Dyslipidemias in drug antiretroviral therapy include increases in total serum cholesterol, particularly an increase in the atherogenic non-high density lipoprotein (non-HDL) cholesterol and triglycerides.⁵ HIV-infected patients may exhibit multiple known risk factors for cardiovascular disease. Of specific concern is the fact that treatment with essential components of HAART, such as non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs), particularly when both are used in combination, is associated with a lipid profile known to increase the risk of coronary heart disease, particularly among older subjects with increased CD4 cell counts and suppressed HIV replication.⁵ In the large DAD multicohort study, hypercholesterolemia and hypertriglyceridemia have been recently shown to be independent predictors of myocardial infarction in HIV-infected patients on HAART.² While several risk factors for dyslipidemia during antiretroviral therapy have been identified, the roles of hepatitis virus coinfections and liver function have been only preliminarily investigated in this regard. In a Spanish cohort, patients coinfecting with hepatitis C virus (HCV) had considerably lower rates of hyperlipidemia than those without HCV, and this effect was particularly evident in those treated with HAART.⁶ A second study showed that HCV-coinfecting patients starting HAART had lower cholesterol levels over time but identical triglycerides.⁷

With the aim of evaluating the effect of HCV infection on the risk of reaching clinically significant serum lipid levels after beginning antiretroviral therapy, in the context of other relevant risk factors for hyperlipidemia, we ana-

lyzed the observational cohort database from a tertiary care center for infectious diseases in Rome, Italy, from 1996–2003. We selected HIV-1-infected patients beginning HAART, defined as any combination of ≥ 3 drugs including at least one PI or NNRTI, with known baseline hepatitis B virus (HBV) and HCV serostatus, and with CD4, HIV RNA, and fasting serum lipids available at baseline and during follow-up, indicated as the first available result in the database. Logistic regression was employed to analyze predictors of categorical outcomes.

Of 907 patients beginning HAART, 225 meeting the above-mentioned criteria were selected for the analysis. At baseline, mean age was 37 years (95% CI, 36–38); 142 (63%) were males; 90 (40%) were injection drug users; 76 (34%) started an NNRTI-based HAART, 145 (64%) a PI-based HAART, while 4 (2%) started a combination of PI plus NNRTI. Mean CD4 cell counts were 314/ μL (95% CI 299–350); mean HIV RNA 4.3 log copies/mL (95% CI 4.15–4.36); mean fasting total cholesterol was 6 mM (177 mg/dL); mean fasting triglycerides 1.4 mM (123 mg/dL); mean alanine aminotransferase (ALT) 32 IU/L; 108 (48%) patients were anti-HCV positive and 21 (9%) were positive for the hepatitis B surface antigen.

After starting HAART, by intention-to-treat analysis, 149 (66%) patients reached HIV RNA <500 copies/mL within week 32. At the first available follow-up (mean 13 weeks, 95% CI 12–15), the National Cholesterol Education Program (NCEP)-defined cutoffs for clinically meaningful dyslipidemia⁸ were overcome in the following numbers: 39 patients (17%) reached total cholesterol >6.2 mM (>240 mg/dL), 63 (28%) had HDL <0.9 mM (35 mg/dL), and 81 (36%) had triglycerides >2.3 mM (200 mg/dL).

At univariate analysis, the following variables were found to be associated with the risk of total cholesterol >6.2 mM: age (for 1 year more, odds ratio [OR] 1.04; 95% CI 1.00–1.10), anti-HCV-positive status (OR 0.22; 95% CI

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TABLE 1. Predictors of Atherogenic Lipid Profile According to NCEP Thresholds (Results From Separate Multivariate Logistic Regression Models)

Variable	Odds Ratio for Showing Atherogenic Lipid Levels (95% CI)		
	Total Cholesterol >6.2 mM	HDL Cholesterol <0.9 mM	Triglycerides >2.3 mM
Age (each 1 year more)	1.06 (1.01–1.11)		
Sex (female vs. male)		0.21 (0.09–0.50)	0.41 (0.22–0.80)
HCV antibody positive vs. negative status	0.19 (0.08–0.48)		
NNRTI-based vs. PI-based HAART		0.49 (0.24–1.03)	1.95 (0.28–1.00)

0.09–10.53), baseline total cholesterol (each 1 mg/dL higher, OR 1.03; 95% CI 1.01–11.05), while age, gender, HBV serostatus, baseline ALT, HIV RNA, and type of therapy were not associated. At multivariate analysis, independent predictors of total cholesterol >6.2 mM were older age and anti-HCV-positive status (Table 1). Univariate predictors of HDL <0.9 mM were NNRTI-based HAART (OR 0.44; 0.22–10.88) and female sex (OR 0.19; 0.09–10.40) but not hepatitis virus serostatus; both variables remained independent predictors of elevated HDL at multivariate analysis (Table 1). Variables associated with triglyceride levels >2.3 mM were older age (OR 1.03; 95% CI 1.00–1.07), female sex (OR 0.36; 95% CI 0.20–0.66), NNRTI-based HAART (OR 0.45; 0.25–0.84), and PI-based HAART (OR 1.95; 1.05–1.60), while hepatitis virus serostatus, baseline ALT and triglyceride levels, and viral load did not show significant associations. Virologic response showed a borderline association with triglycerides above NCEP cutoff (OR 1.88; 0.88–4.00). At multivariate analysis, factors showing an independent negative association with hypertriglyceridemia were female sex and the use of NNRTI-based HAART (Table 1).

This observational cohort study showed in HIV-infected individuals an independent association of HCV coinfection with a reduced risk of clinically relevant elevations of total cholesterol. Older age, male gender, and PI-based HAART as compared with NNRTI-based HAART were other significant predictors of an atherogenic lipid profile.

Our findings confirm and extend previous observations that coinfection with HCV is associated with a lower probability of total cholesterol elevations after HAART initiation. In particular, in HIV/HCV-coinfecting patients, 3 months after HAART initiation, we detected a mean 80% reduction in the adjusted risk of total cholesterol elevations judged clinically relevant by NCEP guidelines. Given that HCV did not show associations with HDL cholesterol levels in this study, it can be indirectly deduced that the lower risk of total cholesterol elevation is related to the non-HDL cholesterol component. While HCV had no influence on triglyceride elevations, NNRTI-based regimens as compared with PI-based as well as female sex showed a reduced probability of low HDL cholesterol and high triglyceride levels: these variables may therefore be associated with a lower cardiovascular risk. Although lipid levels represent only surrogate markers of the cardiovascular risk, factors associated with its elevations should be taken into account when choosing treatment regimens in the individual patient.

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Directly Observed Therapy for HIV Antiretroviral Therapy in an Urban US Setting

To the Editor:

In the United States, the use of antiretroviral therapy (ART) to treat HIV

has led to dramatic reductions in AIDS mortality.¹ However, not all those living with HIV in the United States have experienced the same reductions in mortality. Possible reasons for these differential death rates include tardy diagnosis, poor adherence, and differential access to health care and medications based on race, gender, class, mental illness, and substance abuse histories.²⁻⁵ Barriers to adherence with ART are complex and include a myriad of patient-, regimen-, and system-related factors.⁶ To improve HIV outcomes and reduce medical expenditures, there is a need for effective, sustainable, and replicable strategies to improve ART adherence. Directly observed therapy (DOT) has been used with success in the treatment of tuberculosis; this intervention has been suggested and piloted as a method to improve ART adherence.⁷⁻¹⁰ In Haiti, Partners In Health (PIH) successfully implemented a “DOT-ART” program in 600 patients.¹¹ For the first time, PIH’s Haiti experience has been translated to the urban United States. The Prevention and Access to Care and Treatment (PACT) Project, Boston’s first community-based integrated AIDS prevention and treatment project, initiated a program of “DOT-Plus”—home-based DOT of ART enhanced by case management and counseling. This DOT-Plus program was designed to improve HIV treatment outcomes among patients in whom self-administered ART had failed.

METHODS

DOT-Plus Eligibility Criteria

Within 3 months prior to referral, eligible patients had a CD4 count <350 cells/ μ L and HIV viral load greater than the lowest level of detection of the available assay despite at least 6 months of ART. Patients with new diagnoses of HIV who had not been on ART for at least 6 months were excluded from the pilot study. Eligible patients also met at least two of the following criteria: an AIDS-defining illness, as defined by World Health Organization (WHO) criteria¹² within the past 2 years, hepatitis C, mental illness, as defined by *Diagnostic and Statistical Manual of Mental Disorders IV* criteria¹³ or current prescription of psychiatric medications, active substance abuse within the 30 days prior to referral, or social instability, as defined by poverty (annual income <200% federal poverty guidelines), domestic violence within the 6 months prior to referral, present homelessness, or a lack of a social support network (as defined by lack of a family or friend on whom to call in the event of a medical emergency). In addition, entry into the DOT-Plus program required living within a 20-minute drive of PACT headquarters and a baseline HIV genotype demonstrating a viable once-daily ART regimen.

Selection of Once-Daily ART Regimen

Baseline HIV genotype was performed to ensure susceptibility to once-daily ART. Referring infectious disease specialists selected the ART regimens. Prepackaged pillboxes were delivered to patients on a monthly basis.

Training of DOT-Plus Workers

Two DOT workers were recruited from the community and received 30 hours of training and 10 hours of supervised fieldwork per an established PACT curriculum. (The PACT health promoter training manual will be published shortly.)

DOT-Plus Protocol

These 2 full-time DOT workers supervised once-daily ART doses (as well as other medications) in participants’ homes 7 days per week. Visits ranged from 15–90 minutes depending on the social, cognitive, and medical needs of each participant. The DOT workers collaborated with PACT health promoters who provided the “Plus” component of the DOT-Plus protocol. PACT health promoter activities including management of social crises such as domestic violence and substance abuse, accompaniment to medical and mental health appointments, education about medications and side effect management, and adherence counseling. Education and counseling were delivered weekly according to the PACT health promoter manual. PACT health promoters communicated with DOT-Plus participants at least 3 times per week and made 1 weekly home visit. PACT health promoters and DOT workers had daily phone communication and met weekly to discuss mutual patients.

Outcome Measurement

HIV viral loads and CD4 cell counts were obtained at baseline and 1, 3, 6, 9, and 12 months. Some viral loads were performed using the bDNA assay, and some using the Roche RNA assay. Adherence rates were calculated for observed doses, with the observed dose rate equal to the number of doses observed and kept down/total prescribed doses. Occasional failure to observe doses occurred if patients did not arrive at their scheduled DOT rendezvous. Therefore, “total adherence rates,” or adherence rates for [observed + unobserved but taken and kept down doses]/total prescribed doses, were calculated. An unobserved dose was only counted as having been taken if the pillbox was appropriately empty on the following day, and the patient reported having taken the dose at the appropriate time. If the patient vomited the medications, the dose was recorded as untaken. Patient satisfaction was reported through questionnaires ad-

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ministered by research assistants. Regular communication with referring physicians and chart review enabled gathering of hospitalization and illness data. Health care utilization measures of interest included hospitalization data before and after the DOT-Plus intervention.

Study Timeline

The first cohort of 7 patients was enrolled in July and August 2002, and the second cohort of 8 patients was enrolled in January and February 2003, for a total of 15 patients. Patients enrolled in the DOT-Plus protocol received PACT services until they no longer wished to participate in the program. In this paper, 6-month data are presented for all 15 patients. Twelve-month data are presented for the first cohort only, as the second cohort has not yet reached the 12-month follow-up point.

Statistical Analysis

A 2-sided Wilcoxon sign-rank test was used to determine statistical significance of changes observed in CD4 cell counts and viral load. *P* value <0.05 was considered statistically significant. Intention-to-treat analysis was used. All patients had CD4 and viral load data collected and analyzed, even if they had dropped out of DOT-Plus.

Internal Review Board Approval

Approval was obtained from the Brigham and Women's Hospital Internal Review Board for conducting this study.

RESULTS

Participant Recruitment and Enrollment

In June 2002, we reviewed the PACT cohort and approached 7 patients who had been receiving PACT health promotion services but still met eligibility criteria for participation in the DOT-Plus study. All 7 patients agreed to enroll and began DOT-Plus in July 2002. In December 2002, we opened enrollment to non-PACT patients, who were subject to

the same eligibility criteria as the PACT-referred patients. Area infectious disease specialists referred 20 eligible patients to our project. We approached the 8 patients with the lowest CD4 counts; all 8 agreed to enroll in the protocol.

Participant Characteristics

The 15 DOT program participants had characteristics that have been associated in the literature with nonadherence and increased AIDS morbidity and mortality: nonwhite (*n* = 15), female (*n* = 10), active substance abuse (*n* = 7), clinical depression (*n* = 11), cognitive deficit (*n* = 3). Median viral load at baseline was 121,763 copies/mL (range: 69.0–>500,000 copies/mL), and median CD4 count at baseline was 83 cells/μL (range: 8–298 cells/μL).

Participant Retention

The retention rate for the protocol was 87%, with only 2 study participants dropping out of DOT-Plus, one because of domestic violence and the other due to extreme depression and alcoholism. Relevant outcome data continue to be collected for these 2 patients.

Adherence

For the 13 participants who continue to receive DOT-Plus, observed adherence rates have varied from 63–95%, with an average of 81%. Total adherence rates have ranged from 86–100% with an average of 97%. Reasons for missed doses have included vomiting of pills, patients not being home for DOT visits, or refusal of pills because of nausea, pill fatigue, or potential breach of confidentiality due to guests in the home. The 2 patients who dropped out of DOT-Plus stopped their ART medications completely for the remaining months of follow-up.

Clinical Outcomes

Of the 15 participants, all of them with long histories of ART therapy, most have had dramatic reductions in viral load and increased CD4 counts. Eleven participants have HIV viral loads less

than the lowest level of detection. Using an intention-to-treat analysis (*n* = 15), decrease in median viral load from baseline was $\log_{10} 2.6$ copies/mL (*P* = 0.001) at 6 months. Median CD4 count among the 15 participants increased from 83 cells/μL (range: 8–298 cells/μL) at baseline to 106 cells/μL (range: 11–578 cells/μL) at 6 months (*P* = 0.11). During the 6 months prior to enrollment, there were 10 total hospitalizations with a total length of stay of 52 hospital days for the 15 enrolled patients. Seven hospitalizations were AIDS-related with a total length of stay of 48 days. During the 6 months after enrollment, there were 5 hospitalizations with a total length of stay of 16 days for the 15 enrolled patients. Three hospitalizations were AIDS related, with a total length of stay of 5 days. One hospitalization was related to toxicity of medications (nausea/vomiting) with a total length of stay of 2 days. Thus, we observed a dramatic decrease in the number of hospitalizations and number of hospitalization days within the 6 months following initiation of the DOT-Plus protocol. Further data will be necessary to determine the statistical significance of these findings.

As a secondary endpoint, 12-month data were analyzed for the 7 patients who had reached 1 year in the program at the time of analysis. Using an intention-to-treat analysis, decrease in median viral load from baseline at 12 months was $\log_{10} 2.96$ copies/mL (*P* = 0.02). Median CD4 count increased to 192 cells/μL (range: 16–262 cells/μL) (*P* = 0.22).

DISCUSSION

Preliminary results from this community-based DOT program suggest that daily DOT of a once-daily ART regimen among a challenging urban population is both acceptable to patients and feasible. Retention rates were high and many patients who are still receiving DOT-Plus at 15 months are requesting ongoing services. Although small sample size and short follow-up time limit our conclusions, preliminary results indicate that

>90% total adherence rates can be achieved in the majority of participants, and that clinically important improvements in CD4 and viral load can be observed within a relatively short period. In addition, there is a decrease in the number of total hospitalizations and length of stay comparing the 6 months prior to and after enrollment in the DOT-Plus protocol.

We applied the success of DOT of ART in rural Haiti to the urban United States, where HIV disease is increasingly associated with poverty and marginalization. DOT-Plus was offered to patients with advanced HIV disease in whom conventional self-administered therapy had already failed. Among these AIDS patients, most with some degree of ART resistance, 11 of 15 previously unsuppressed patients subsequently achieved undetectable viral loads. These rates of viral suppression exceed those observed in sociologically similar patient populations. In one study, Lucas et al.¹⁴ reported that only 37% of patients attending an inner city comprehensive HIV clinic had viral suppression 7–14 months after ART initiation.

CONCLUSION

This study evaluates the feasibility of DOT for a subset of difficult-to-reach patients in whom unsupervised therapy for advanced HIV disease has failed. We have shown that a DOT-Plus program is feasible and acceptable among a group of patients facing myriad social problems. Preliminary results suggest clinical improvement and reduced hospitalizations. A randomized controlled study with a greater number of patients is needed to demonstrate effectiveness, long-term participant retention, viral suppression and resistance repercussions, cost effectiveness, and sustainability of this complex adherence intervention. In addition, the relative contribution of the DOT vs. the case management components of the DOT-Plus intervention as well as the eventual transition to successful self-administration will need to be evaluated.

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HIV Protease Inhibitors Increase Adiponectin Levels in HIV-Negative Men

To the Editor:

Lower levels of adiponectin, a hormone secreted by adipose tissue, have been associated with insulin resistance and increased visceral adipose tissue.^{1,2} Adiponectin has been shown to directly and rapidly decrease endogenous glucose production and improve glucose metabolism and fatty acid utilization in the liver and skeletal muscles *in vivo*.^{3,4} The relation of adiponectin to insulin sensitivity is independent of changes in other known adipocytokines, including leptin.⁵ Adiponectin is inversely associ-

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ated with visceral and total adipose mass, as well as insulin sensitivity.

Both HIV-induced lipoatrophy and lipohypertrophy are associated with lower adiponectin levels,⁵ but the cause is unknown. HIV protease inhibitors have been studied as a possible cause of lower adiponectin levels. Although preliminary data found that protease inhibitors caused decreased adiponectin expression in fat cells *in vitro*,⁶ one group recently reported increased adiponectin levels during indinavir treatment in HIV-negative men *in vivo*.^{7,8} They have postulated that indinavir induced the increase in adiponectin levels due to either induction of insulin resistance or endothelial dysfunction.

Previously, we reported that the protease inhibitors, indinavir and lopinavir/ritonavir, have different metabolic effects in HIV-negative men.^{9,10} Whereas indinavir induced insulin resistance with no effect on lipid metabolism, lopinavir/ritonavir increased fasting triglycerides and free fatty acids but had little or no effect on insulin sensitivity during the euglycemic hyperinsulinemic clamp. Indinavir and other protease in-

hibitors can induce insulin resistance without changes in body fat.⁹⁻¹¹ Therefore to assess the effects of 2 different protease inhibitors on adiponectin levels *in vivo*, we measured adipocytokine levels in indinavir- and lopinavir/ritonavir-treated subjects.

As previously reported, HIV-negative men were treated with indinavir 800 mg 3 times daily⁹ or lopinavir 400 mg/ritonavir 100 mg twice¹⁰ daily. Fasting lipid and lipoprotein profiles, insulin sensitivity (euglycemic hyperinsulinemic clamp), and body composition were measured before and at the end of 4 weeks of treatment. Adiponectin and leptin levels were measured by radioimmunoassay (Linco Research, Inc., St. Charles, MO) in serum obtained after overnight fasting; serum samples were stored at -70°C . The data met normality assumptions and paired *t* tests were performed using Sigma Stat v. 2.03 (SPSS, Inc., San Rafael, CA). Data are presented as mean \pm SEM. Two-tailed *P* value <0.05 was considered statistically significant.

Insulin-mediated glucose disposal per unit of insulin (M/I) decreased by

19% in response to indinavir with no change in lipid or lipoprotein profiles (Table 1). During lopinavir/ritonavir treatment, fasting triglycerides and free fatty acids increased with no change in insulin-mediated glucose disposal. Abdominal visceral and subcutaneous adipose tissue measured by CT scan as well as total, appendicular, or trunk fat by dual-energy x-ray absorptiometry did not change after either treatment.

Adiponectin levels increased with indinavir and to an even greater extent with lopinavir/ritonavir. In contrast, leptin levels did not change after either treatment. There was no correlation between individual changes in body composition and changes in adiponectin levels.

The observation that serum adiponectin levels increased during treatment with both indinavir and lopinavir/ritonavir has several implications. First, the increased adiponectin levels induced by indinavir and lopinavir/ritonavir treatment cannot explain the lower levels of adiponectin found in patients with HIV-associated lipohypertrophy and lipoatrophy, although many were on protease inhibitors. These data raise the possibility

TABLE 1. Metabolic Parameters at Baseline and at the End of 4-Week Treatment with Indinavir and Lopinavir/Ritonavir

	Indinavir			Lopinavir/Ritonavir		
	Baseline	4-Week Treatment	<i>P</i> Value	Baseline	4-Week Treatment	<i>P</i> Value
Insulin-mediated glucose disposal (mg/kg \square per $\mu\text{U}/\text{mL}$ insulin)*	10.4 \pm 1.4	8.6 \pm 1.2	0.009	15.9 \pm 2.1	16.2 \pm 2.3	0.81
Triglycerides (mmol/L)	1.4 \pm 0.2	1.7 \pm 0.4	0.22	0.9 \pm 0.2	1.6 \pm 0.4	0.007
Free fatty acids (mmol/L)*	0.30 \pm 0.02	0.22 \pm 0.04	0.31	0.33 \pm 0.04	0.43 \pm 0.06	0.001
Adipose tissue by CT scan						
Subcutaneous (mm ²)*	14,762 \pm 1,577	14,629 \pm 1,651	0.72	17,629 \pm 2,025	16,922 \pm 2,371	0.29
Visceral (mm ²)*	8896 \pm 1,865	9449 \pm 2,178	0.28	8950 \pm 1,933	8560 \pm 2,094	0.11
DEXA scan						
Total fat tissue (kg)*	15.8 \pm 1.9	15.2 \pm 1.9	0.01	16.8 \pm 1.9	16.6 \pm 1.2	0.31
Appendicular fat (kg)*	6.7 \pm 0.7	6.5 \pm 0.6	0.08	8.5 \pm 0.7	8.4 \pm 0.7	0.6
Trunk fat (kg)*	8.3 \pm 1.2	7.9 \pm 1.2	0.03	7.6 \pm 1.3	7.4 \pm 1.4	0.38
Adiponectin ($\mu\text{g}/\text{mL}$)	9.5 \pm 1.6	10.7 \pm 1.8	0.05	11.0 \pm 1.5	14.0 \pm 2.1	0.005
Leptin (ng/mL)	4.6 \pm 1.0	4.1 \pm 0.8	0.18	4.0 \pm 0.9	3.8 \pm 0.9	0.3

Data are mean \pm SEM. All *P* values are by paired *t* test (*n* = 10).

*Previously reported by Shankar et al.⁸ and Noor et al.⁹

DEXA, dual-energy x-ray absorptiometry.

that body composition changes per se may be the underlying cause of the decrease in adiponectin levels in HIV-associated lipodystrophy, although a role for drugs other than protease inhibitors cannot be ruled out. Secondly, the increase in adiponectin induced by these 2 protease inhibitors in vivo contrasts with the decrease in adiponectin expression in 3T3 adipocytes acutely treated in vitro with indinavir, ritonavir, saquinavir, nelfinavir, zidovudine, or stavudine.⁶ Third, other protease inhibitors should be studied for their effects on adiponectin levels. Finally, because adiponectin levels are increased by both indinavir, which induces insulin resistance, and by lopinavir/ritonavir, which has little or no effect on insulin sensitivity, induction of insulin resistance is unlikely to explain the increased adiponectin levels seen with both protease inhibitors. Thus these data argue against the hypothesis that indinavir-induced insulin resistance is the cause of the increased adiponectin levels.⁷

We cannot rule out the possibility that induction of adiponectin blunted the appearance of insulin resistance. We also found that the increase in adiponectin levels occurred in the absence of changes in subcutaneous or visceral fat or leptin. The induction of adiponectin independent of changes in body fat mass is interesting with regard to thiazolidinedione therapy for HIV lipodystrophy. Thiazolidinediones have been shown to increase adiponectin levels, raising the possibility that adiponectin mediates some of the effects of thiazolidinediones on glucose

metabolism. Recent data¹² have indicated the thiazolidinediones also raise adiponectin levels in patients with HIV lipodystrophy and insulin resistance without major changes in body fat mass. The cause for increased adiponectin levels during protease inhibitor treatment remains to be elucidated. Whether protease inhibitors exert their action on adipose or endothelial cells is unclear. Further studies identifying the mechanism of protease inhibitor-induced adiponectin increase are needed.

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