Delayed Sample Processing Leads to Marked Decreases in Measured Plasma IL-7 Levels

To the Editor:

Interleukin-7 (IL-7) is a cytokine that plays a critical role in lymphocyte homeostasis. Produced by stromal cells, it stimulates thymopoiesis by providing survival signals to early thymocyte progenitors[1] and also stimulates the proliferation of lymphocytes in the periphery.[2,3] Several investigators have demonstrated increased IL-7 levels in CD4 lymphopenic states, such as during HIV infection or after chemotherapy.[4,5] IL-7 levels have been reported in both plasma and serum, but it is not clear whether the levels from these 2 sources differ or how the different processing techniques may affect them.

We compared serum and plasma IL-7 levels from healthy volunteers. Samples were either processed immediately (within 15 minutes of blood draw) or 2 to 4 hours after blood draw. Blood for plasma was collected in tubes containing EDTA and was centrifuged; plasma was removed, aliquoted, and frozen at −20° C. Blood for serum was collected in serum collection tubes and allowed to clot on ice either immediately or 2 to 4 hours after blood draw. After clotting overnight, the tubes were centrifuged; serum was removed, aliquoted, and frozen at −20° C.

Blood was collected from 10 healthy volunteers who presented consecutively over 3 days to the National Institutes of Allergy and Infectious Diseases intramural AIDS research clinic. All participants were enrolled on institutional review board–approved study protocols and gave informed consent. IL-7 levels were measured in plasma and serum using a high-sensitivity enzyme-linked immunosorbent assay (ELISA; R&D Systems, Inc, Minneapolis, MN) according to manufacturer's recommendations. Mean values were compared using paired *t* tests.

As shown in Figure 1, the mean IL-7 levels from immediately processed plasma (10.75 pg/mL) and serum (8.05 pg/mL) were not significantly different ($P = 0.17$); however, they were significantly different if processing was delayed (mean, 0.75 vs. 9.31 pg/mL, respectively; $P < 0.001$). The measured level of IL-7 in plasma was significantly lower if processing was delayed ($P < 0.001$), whereas serum levels did not change significantly ($P = 0.51$).

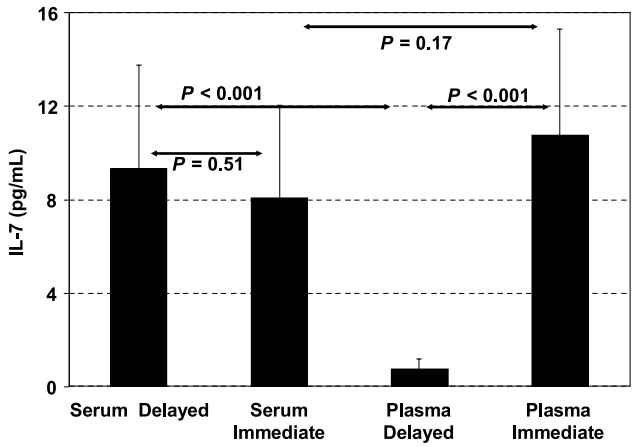

FIGURE 1. Mean plasma and serum IL-7 levels. The samples underwent either immediate processing (within 30 minutes of blood draw) or delayed processing (within 2 to 4 hours of blood draw). Error bars indicate SD.

These results show that although there is no significant difference between plasma and serum IL-7 levels when samples are processed immediately, there is a marked difference in these levels when there is a delay in processing. The difference that results from delayed processing seems only to affect plasma levels of IL-7, whereas levels in serum are stable. Both serum and plasma levels from samples that were processed immediately were higher than the mean values reported by the ELISA kit manufacturer for healthy volunteers (serum: mean, 2.2 (range, 0.27–8.7) pg/mL; EDTA plasma: mean, 2.2 (range, 0.66–9.2) pg/mL.[6] We cannot exclude the possibility that these higher levels may be related to the method of processing of the samples.

There are several possible mechanisms for the decreased levels seen in plasma samples after delayed processing. There may be degradation or modification of IL-7 due to the presence of proteins found in plasma, but not in serum, leading to a lack of recognition by the antibodies present in the IL-7 ELISA. Likewise, there may be binding of IL-7 to proteins or to cellular receptors. It is important to note that these experiments were performed with plasma samples collected with EDTA and with the use of a single type of ELISA kit; these results cannot be extrapolated to the use of other anticoagulants or ELISA kits.

These results clearly show that plasma and serum levels cannot be used interchangeably (e.g., when making comparisons between groups), unless samples have been processed immediately after collection and appropriate comparisons for the specific laboratory and methodology have been performed. It is also important to standardize the collection techniques when IL-7 levels are being measured, particularly in multicenter clinical trials. Finally, it is equally important to provide details of collection and processing techniques when IL-7 levels are reported in a publication.

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Polymorphism of the Protease and Reverse Transcriptase and Drug Resistance Mutation Patterns of HIV-1 Subtype B Prevailing in China

To the Editor:

HIV replication can be markedly inhibited by highly active antiretroviral therapy, whereas some mutations in the genes of reverse transcriptase (RT) and protease, which endow the virus with drug resistance ability, are considered a major cause of treatment failure. Although many studies have focused on characterization of the polymorphisms or drug resistance mutations of protease and RT gene, HIV-1 subtype B isolates of North America and Europe were studied in most cases. In this study, we analyzed the polymorphism of protease and RT gene in HIV-1 subtype B prevailing in China. The treatment-associated resistance mutation spectrum of HIV-1 RT gene during RT inhibitors (RTIs) therapy was also studied.

The subjects of this survey were from 4 midland provinces of China (Anhui, Henan, Shanxi, and Sichuan) where the HIV-1 subtype B is mostly prevalent; all of the samples were collected in 2004. RNA was extracted from plasma samples, and the sequences of protease and RT were obtained by reverse transcription nested polymerase chain reaction and sequencing. All sequences belonging to subtype B were compared with the subtype B consensus sequence in Stanford HIV Drug Resistance Database (http://hivdb.stanford.edu) using the HIVdb software (version, 4.1.2). The χ^2 tests or the Fisher exact tests were used to compare the differences of the proportion of the amino acid substitution at each position between different groups or to determine the correlations between different mutation positions.

One hundred seventy-four sequences of protease and 197 sequences of RT from 197 subjects infected with HIV-1 subtype B were obtained. All of the 197 participants were of Han nationality, predominantly aged from 30 to 50 years (81.7%). The percentage of men was 50.8%. The large majority of patients indicated that they were most likely infected with HIV through blood donation (89.8%). All of the patients were treatment-naive of protease inhibitors (PIs). Seventy-one patients (36.0%) never received antiretroviral therapy; 126 other patients (64.0%) experienced RTIs therapy; 55 patients (27.9%) used a regimen of azidothymidine (AZT), didanosine (ddI), and nevirapine (NVP); 66 patients (33.5%) used a regimen of stavudine (d4T), ddI, and NVP; 4 patients (2.0%) used a regimen of AZT, lamivudine (3TC), and NVP; and 1

TABLE 1. The Incidence of Amino Acid Substitution in Protease of HIV-1 Subtype B Isolated From 174 HIV/AIDS Patients Never Treated With PIs

Position	The Amino Acid of Clade B Consensus	Subtype B in China	
		Substituted Amino Acid and Proportion (%)	Total Substitution Proportion (%)
5	L	P (0.6), X (0.6)	1.1
10	L	I (3.4)	3.4
12	T	A (4.6), K (0.6), P (8.6), S (1.1)	14.9
13	I	V (4.0)	4.0
14	K	R (7.5)	7.5
19	L	E (0.6), I (1.7), V (8.0)	10.3
31	T	P (0.6), S (0.6)	1.1
33	L	I (0.6), V (0.6)	1.1
35	E	D (79.9), K (1.1)	81.0
37	N	D (0.6), H (0.6), S (1.1)	2.3
41	R	K (10.9)	10.9
43	K	R (1.7)	1.7
51	G	E (0.6), V (0.6)	1.1
57	R	K (10.3)	10.3
60	D	E (1.7)	1.7
61	Q	E (2.9), H (0.6), N (0.6), S (0.6)	4.6
62	I	V (20.7)	20.7
63	L	C (0.6), P (90.2), S (6.9), T (2.3)	100.0
64	I	L (1.7), M (1.7), V (1.7)	5.2
67	C	S (0.6), W (0.6), Y (0.6)	1.7
69	H	P (1.1), N (0.6)	1.7
70	K	N (0.6), R (1.7)	2.3
71	A	I (0.6), T (20.7), V (10.9)	32.2
72	I	E (17.2), V (77.0)	94.3
76	L	F (0.6), I (0.6)	1.1
77	V	I (96.6)	96.6
93	I	L (91.4), V (0.6)	92.0

patient (0.5%) changed the regimen from d4T, ddI, and NVP to AZT, 3TC, and efavirenz (EFV).

In this study, polymorphism is the variation from the consensus sequence within the subtype B, arbitrarily defined as position-specific amino acid differences occurring in more than 1% of protease or RT sequences isolated from those patients who are treatment-naive of PIs or RTIs. If the proportion of an amino acid is more than 50%, then this amino acid is considered to be the inherent amino acid at this position.

We found that polymorphisms occurred at 27 codons (27.3%) of the total 99 amino acids of protease (Table 1); the proportions of amino acid substitution at positions 63 (100.0%), 77 (96.6%), 72 (94.3%), 93 (92.0%), and 35 (81.0%) were all more than 50%; the L63P (90.2%), V77I (96.6%), I72V (77.0%), I93L (91.4%), and E35D (79.9%) were the inherent amino acid of the protease of the HIV-1 subtype B strains prevailing in China compared with the strains prevailing in America and Europe. In addition, the proportions of amino acid substitution were between 20% and 50% at the positions 71 (32.2%) and 62 (20.7%).

Positions 63, 71, 77, and 93 are also the polymorphic positions in HIV-1 subtype B prevailing in America and Europe, and these positions are considered as the secondary drug resistance mutation positions. Previous studies indicated that, in America and Europe, about 55% of isolates have amino acid substitution (amino acid other than L) at positions 63, about 25% at positions 77 and 93, and about 5% at position 71 in untreated persons; in heavily treated patients, those proportions reached 90% at position 63, 40% at positions 77 and 93, and 70% at position 71.[1,2] In this study, the proportions of L63P, V77I, and I93L in untreated patients were even higher than those in heavily treated patients described previously. In addition, the proportion of amino acid substitutions at protease position 71 was higher than that of subtype B prevailing in America and Europe.

Although secondary mutations do not significantly alter the susceptibility of the PIs, these mutations, which exist before the emergence of a primary mutation, may alter the rate of development of drug resistance or the subsequent evolution of mutations.[3] It is reported that a relatively poorer response to therapy was associated with high-baseline protease polymorphism and with the presence of I93L and A71V/T at baseline.[4] Therefore, the high frequencies of baseline amino acid substitutions at positions 63, 71, 77, and 93 of protease in HIV-1 subtype B prevailing in China may have potential influence on the curative effects of PIs that will be introduced in antiretroviral therapy of China hereafter.

In this study, 71 RT sequences were obtained from untreated patients. Seventy-four (24.7%) of the first 300 amino acid positions of RT were found to be polymorphic. The proportions of amino acid substitution at positions 278 (100.0%), 162 (95.8%), 200 (90.1%), 135 (83.1%), and 277 (74.2%) were more than 50%; the Q278E (100%), S162C (93.0%), T200A (81.7%), I135V (69.0%), and K277R (74.2%) were the inherent amino acid of RT. In addition, relatively high proportions of amino acid substitution were found at positions 211 (31.0%) and 293 (24.2%). All amino acid substitutions previously mentioned have not been reported to be associated with the RTIs resistance, except for position 135. Previous study indicated that those strains containing the I135M/L/T substitution in combination with the L283I substitution showed decreases in susceptibility to all 3 of the nonnucleoside reverse transcriptase inhibitors (NNRTIs).[5] In our study, although the total proportion of I135L/T is 9.8%, the polymorphism at position 135 would not influence the susceptibility of HIV-1 to drugs because there was no L238I existing together with I135L/T. In this study, only 3 isolates from 71 treatment-naive patients harbored 3 nucleoside reverse transcriptase inhibitors (NRTIs) resistance–associated mutations (A62V, Y115F, and T215A); no NNRTIs resistance mutation was found. These results demonstrated that the frequency of baseline RTIs resistance mutations was very low among these treatment-naive patients.

In the RT sequences from 126 patients undergoing RTIs therapy, we found 6 kinds of NRTIs resistance mutations at 4 amino acid positions: K65R (3.2%), D67G (1.6%), D67N (1.6%), L74V (4.8%), T215F (0.8%), and T215Y (2.4%). In contrast, we found 10 kinds of NNRTIs resistance mutations at 7 amino acid positions: K101E (2.4%), K103N (34.1%), K103S (1.6%), V106A (4.0%), Y181C (23.8%), Y188C (0.8%), Y188L (0.8%), G190A (15.1%), G190S (1.6%), and K238T (1.6%). Notably, the proportions of K103N, Y181C, and G190A mutations were significantly higher in treated patients than in untreated patients ($P = 0.000$, 0.000, and 0.001, respectively). These results indicated that the emergence of NNRTIs resistance mutations is also an important problem during antiretroviral therapy in China.

To find whether there were novel mutations in RT associated with anti-HIV therapy, the proportions of amino acid substitution at each mutation position were compared between RTI-treated and untreated groups. We found that position 221 was highly conserved in HIV-1 isolates from untreated group (proportion, 0/71), whereas amino acid substitution at this position occurred in treated patients with higher proportion (14/126). Moreover, as the most frequent amino acid substitution of this position, the proportion of H221Y was significantly higher in RTI-treated patients (13/126 [10.3%]) than that in untreated patients ($P = 0.012$), which indicated that H221Y mutation was associated with RTIs treatment.

Furthermore, we compared the proportions of H221Y between 2 groups with and without K103N or Y181C mutations—the 2 most frequent drug resistance mutations in RT after RTIs treatment in our study. The proportion of H221Y in the group with Y181C was significantly higher than that in the group without Y181C (40.0% vs 1.0%, $P = 0.000$). However, there was no significant difference in proportion of H221Y between 2 groups with and without K103N (14.0% vs 8.4%, $P = 0.511$).

H221Y is a novel therapy-related mutation first reported by Gonzales[6] in 2003. Gonzales et al proposed that H221Y was associated with NRTI therapy. But this proposition is discrepant with the results of a recent study in which the H221Y was indicated to be associated with NNRTI resistance.[7] In our study, the H221Y mutation was found to be correlated with Y181C mutation ($\phi = 0.545$), whereas Y181C

has been reported to be NNRTIs resistance mutation; thus, we speculate that the H221Y mutation is more likely associated with the NNRTIs resistance.

In summary, there are inherent amino acids at some positions of protease and RT of HIV-1 subtype B strains prevailing in China compared with the strains in America and Europe. As the secondary PIs resistance mutations, the high frequencies of L63P, V77I, and I93L may have potential influence on the curative effect of PIs that will be used in China. The mutations K103N, Y181C, and G190A were the 3 most prevalent drug resistance mutations after antiretroviral therapy in China, and H221Y was a novel mutation more likely associated with the NNRTI therapy.

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Depressive Symptoms Predict Increased Incidence of Neuropsychiatric Side Effects in Patients Treated With Efavirenz

To the Editor:

Efavirenz (EFV), a nonnucleoside reverse transcriptase inhibitor (NNRTI) of HIV-1, is associated with neuropsychiatric central nervous system side effects not always seen with other members of the NNRTI class. Although the mechanism of action responsible for these side effects has not been elucidated, EFV has a 60% homology with antidepressants, with autonomic and cognitive adverse events.[1,2] The neuropsychiatric side effects secondary to EFV include dizziness, confusion, stupor, impaired concentration, amnesia, hallucinations, abnormal dreams, and insomnia.[3] Most of the EFV-related neuropsychiatric side effects occur within the first several days of treatment and tend to resolve within the first 6 to 10 weeks of therapy; however, they may be serious and can include anxiety, depression, and suicidal ideation.[4,5] We were particularly interested in evaluating neuropsychiatric side effects in patients able to tolerate an EFV-containing regimen for more than 4 weeks while still experiencing neuropsychological side effects. By identifying an association between depression history and neuropsychological symptoms, we hoped to better predict the development and progression of long-term EFV-related neuropsychiatric side effects, which could negatively affect treatment adherence.[6,7]

Of all patients in the clinic over the set period, medical records of 110 eligible patients receiving EFV-based therapy from 6 participating private HIV practices in the San Francisco Bay area were reviewed to examine the number of neuropsychiatric side effects and the number of patients discontinuing EFV because of these effects. Baseline demographics, $CD4^+$ cell counts, and viral loads were recorded along with concurrent medications. Patients with documented or suspected use of cocaine, methamphetamines, ecstasy, ketamine, γ-hydroxybutyrate, or heroin were excluded from this statistical analysis. Patients not receiving EFV-based antiretroviral therapy (ART) for more than 4 weeks were also excluded. A history of depression was defined as one or a combination of the following factors diagnosed and resolved more than 6 months before starting an EFV-containing regimen: (*a*) patient history of depressive symptoms, (*b*) patient under the care of a psychiatrist/therapist, and (*c*) patient under antidepressive treatment. The length of time the patient remained on EFV before discontinuation was noted, as was the viral load and $CD4^+$ count at the time of drug discontinuation. Current depression was defined as documented depression in the patient's chart within 6 months of starting EFV. Charts were further examined to identify the occurrence and magnitude of EFV-related depressive symptoms during EFV treatment. Using a specialized evaluation questionnaire as per the Sustiva (Bristol-Myers Squibb, New York, NY) package insert, developed to standardize data collection, EFV-related neuropsychiatric symptoms were graded on a scale of 1 to 5 by frequency of occurrence: grade 1, never experienced; grade 2, rarely (0–1 day a week); grade 3, sometimes (2–4 days a week); grade 4, very often (4–5 days a week); and grade 5, most of the time (6–7 days a week).

For the primary analysis, the number of discontinuations for each group was tabulated, and a relative risk (RR) and 95% confidence interval for the odds ratio were calculated. The *P* value for the difference between odds ratio was then determined. For the secondary depression subcategory analysis, median scores

for each of the previously defined groups were calculated, with the difference in the median values assessed by a Wilcoxon rank sum test.

The charts of 110 patients were screened. Most of the patients were men having sex with men (99%), with a median age of 43 years. All statistical analyses were performed on 101 remaining patients unless otherwise stated. The median baseline $CD4^+$ count was 336 cells/mm^3, and the median viral load was 25,000 copies/mL, both with wide ranges. Fifty patients (50%) had a history of depression; of these, 56% were currently depressed. History of depression was further divided into subcategories: reported by patient (n = 5), depression requiring medication (n = 30), and depression requiring psychotherapy (n = 19). Four patients reported depression requiring both medication and psychotherapy in the past; one discontinued EFV-based ART. Relative risk for current depression was significantly higher for patients with any history of depression than for patients without such a history (RR, 31.2; 95% CI, 6.81–142.6, $P < 0.0001$). Eighteen percent of patients with a history of depression discontinued EFV because of treatment-related symptoms of depression (RR, 11; 95% CI, 1.3–90.2, $P = 0.0079$), whereas only 2% of patients who did not have a history of depression at any time discontinued EFV (RR, 10.0, 95% CI, 1.2–82.6, $P = 0.016$). All patients with a history of depression and/or current depression developed at least one subcategory depressive symptom of grade 2 severity or higher. All depression subcategories associated with EFV, excluding the "unintentional weight loss of more than 5%" subcategory and the "suicidal ideation/attempt" subcategory, were statistically significantly higher ($P \leq 0.0009$) in patients with a history of depression than in patients without such a history.

Our study revealed an overall incidence of neuropsychiatric symptoms of any kind while on an EFV-containing regimen of 30%, with a 10% discontinuation rate due to EFV-associated neuropsychiatric side effects at or after 4 weeks of treatment. Disparity exists between registration/investigational studies and clinical studies performed within primary HIV practices. It has been suggested that in "real world" settings, there is a much greater likelihood that these symptoms will occur because of unrecognized additional pressures, patient risk-taking, and comorbidities that could negatively affect a patient's outcome.[8]

Discontinuation of therapy did not appear to be related to virological failure, as 40% of the patients discontinuing EFV therapy after 4 weeks continued to have viral loads less than 50 copies/mL. If these results are confirmed, this component of treatment failure could potentially be anticipated and adjusted for. Our results tend to support the complex nature of "treatment success" and show clearly that one can continue to "succeed" virologically while "failing" in some other necessary requirement of ART. It should be noted that the use of alcohol and marijuana was high in our study, but no association was found between their use and the incidence of depression or treatment discontinuation. The prevalence of other documented recreational drug use was too low to allow analysis of any potential associations.

If depressive symptoms cannot be tolerated by the patient or ameliorated by psychotherapy, alteration of the ART regimen may be warranted. Other NNRTIs such as nevirapine and protease inhibitors such as lopinavir/ritonavir have not been associated with neuropsychiatric effects of the same severity as those seen with EFV and yet have been shown to have comparable efficacy.[9,10]

Limitations of this study include the fact that this was a retrospective chart review, and diagnosing past depression based on chart documentation may either overestimate or underestimate rates of depression. Moreover, it failed to clarify specific diagnoses, such as major depression versus bipolar depression versus dysthymia, etc, and men having sex with men was the only subpopulation well represented in this sample. Future research that controls for other variables and has a comparison group of patients not taking EFV would help establish whether EFV is the direct cause of new depressive symptoms.

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Further Information on the Administration of H_2-Receptor Antagonists With Atazanavir

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To the Editor:

Further data on the use of atazanavir (ATV) with H_2-receptor antagonists and proton pump inhibitors (PPIs) have become available since the letter published by Drs. Homayoun Khanlou and Charles Farthing.[1] Previously, ATV and PPI interaction studies demonstrated that ATV exposures were reduced by more than 70%, leading to a recommendation that PPIs should not be used concomitantly with ATV.[2,3] Studies to evaluate other dosing strategies with ATV and PPIs are planned. More recently, data have become available on the interaction between ATV and H_2-receptor antagonists, indicating that reduced exposures to ATV resulting from an interaction with H_2-receptor antagonists may be attenuated by concurrent use of low-dose ritonavir (RTV) or by temporal separation from an H_2-receptor antagonist.[4]

In 2 drug interaction studies of 108 HIV-negative subjects, the effect of the commonly used H_2-receptor antagonist famotidine at 40 mg twice daily on ATV at 400 mg and ATV/RTV at 300/100 mg was evaluated.[4] Relative to a control arm of ATV at 400 mg alone, coadministration of famotidine at 40 mg twice daily decreased ATV exposures by approximately 40%. Temporal separation (dosing ATV 10 hours after and 2 hours before the famotidine dose) attenuated the reduction in ATV exposures. Furthermore, ATV at 300 mg coadministered with RTV at 100 mg led to a 79% and 4.5-fold increase in area under the curve and C_{min}, respectively, with C_{max} values similar to ATV at 400 mg alone.

Relative to a control arm of ATV/RTV at 300/100 mg alone, coadministration with famotidine at 40 mg twice daily modestly reduced ATV exposures by 18%, 14%, and 28% for area under the curve, C_{max}, and C_{min}, respectively. These exposures were similar to an antiretroviral regimen of ATV/RTV with 2 nucleosides including tenofovir, the efficacy of which has been established in a well-controlled study in HIV-infected subjects relative to a standard of care regimen.[5] Thus, ATV/RTV at 300/100 mg may be given concomitantly in combination with H_2-receptor antagonists. Atazanavir/RTV at 300/100 mg is recommended with H_2-receptor antagonists, although the reductions in ATV exposures with maximal doses of famotidine could be overcome by increasing the dose of ATV to 400 mg while keeping the dose of RTV steady at 100 mg. Atazanavir/RTV at 400/100 mg is not recommended because there is a potential for increased risk of ATV intolerability, resulting from increased ATV exposures that could occur with less potent, lower doses, or sporadic dosing of H_2-receptor antagonists in combination with higher doses of ATV. To further minimize any reduction in ATV exposures, an alternative to simultaneous dosing of H_2-receptor antagonists with ATV/RTV at 300/100 mg is temporal separation, which may be attained by administering ATV/RTV at 300/100 mg at least 10 hours after and at least 2 hours before an H_2-receptor antagonist; temporal separation should be considered in treatment-experienced patients.

In summary, although H_2-receptor antagonists increase intragastric pH and have been shown to reduce ATV absorption, H_2-receptor antagonists are less potent than PPIs in suppressing intragastric acid secretion and may be used with ATV. Atazanavir/RTV at 300/100 mg may be administered concomitantly with H_2-receptor antagonists. Alternatively, ATV at 400 mg or ATV/RTV at 300/100 mg may be temporally separated by administering ATV at least 10 hours after and at least 2 hours before an H_2-receptor antagonist to minimize the interaction; temporal separation of ATV/RTV at 300/100 mg should be considered in treatment-experienced patients. Local prescribing information may vary; therefore, clinicians using ATV with H_2-receptor antagonists should consult their local package inserts for specific information. Additional studies to evaluate alternative dosing strategies with ATV and H_2-receptor antagonists are ongoing.

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The Correlation Between Prevalence of Oral Manifestations of HIV and $CD4^+$ Lymphocyte Counts Weakens With Time

To the Editor:

Oral manifestations of HIV (OMHs) have been well documented since the onset of the HIV pandemic as early markers of HIV infection and

TABLE 1. Partial Correlations (Controls for Variable 1); Pearson Correlations (Variables 2 and 3)

Variable 1	Variable 2	Variable 3	Partial *r* (sig)	Correlation (sig)
Subjects with a documented HIV infection shorter or equal than 10 years (group 1)				
Smokes	Normal examination	CD4	0.263 (0.007)	0.270 (0.006)
Smokes	No. lesions	CD4	−0.280 (0.004)	−0.286 (0.003)
No. cigarettes smoked per day	No. lesions	CD4	−0.290 (0.003)	
HAART	Normal examination	CD4	0.206 (0.061)	
Subjects with a documented HIV infection longer than 10 years (group 2)				
Smokes	Normal examination	CD4	0.086 (0.324)	0.093 (0.470)
Smokes	No. lesions	CD4	−0.097 (0.264)	−0.104 (0.231)
No. cigarettes smoked per day	No. lesions	CD4	−0.111 (0.201)	
HAART	Normal examination	CD4	0.144 (0.174)	

progression.[1] OMHs are commonly diagnosed and therefore contribute significantly to HIV-related morbidity. Oral candidiasis (OC) and oral hairy leukoplakia, 2 lesions associated with fungal and viral pathogens, respectively, are the most frequently occurring OMHs. Other OMHs, such as human papillomavirus-related warts, aphthous-like ulcers, and Kaposi sarcoma, have also been extensively reported.

The risk of developing an OMH correlates significantly with a decreasing peripheral CD4 count, a marker of HIV disease progression.[2] Therefore, although the treatment of specific OMHs has been reasonably successful, it is evident that the most successful therapeutic approach is to prevent or reverse the underlying primary immunodeficiency disease and thereby prevent OMHs.

Unsurprisingly, the introduction of highly active antiretroviral therapy (HAART) has resulted in a significant decrease in the prevalence of opportunistic diseases including that of OMHs. Seemingly in contradiction with those findings, despite a marked improvement in CD4 cell counts, an increased prevalence in oral warts has been noted by some investigators.[3] Taken together, the epidemiology of OMHs in the HAART era seems to indicate that OMHs are, as a whole, less frequent, but that new and poorly understood paradoxes are emerging. It has been suggested that those apparent paradoxes could be explained at least partially by an incomplete functional reconstitution of the immune system as a result of HAART[4] or by a transient deterioration of immune function referred to as the immune reconstitution syndrome.[5] The quantification of peripheral CD4 T lymphocytes would then not accurately reflect the functional status of the immune system against all opportunistic pathogens and diseases.

We hypothesized that in patients with less than 10 years of diagnosed HIV infection, a lower CD4 count would be associated with an increased risk for OMHs, whereas this association may not exist for the subjects with a longer history of diagnosed HIV infection. The target population for our study was the Ryan White Dental Clinic at the University of Illinois at Chicago. We stratified the subjects into 2 groups, a group with a history of HIV infection shorter than 10 years (group 1) who benefited from HAART throughout their HIV infection and a group with a history of HIV infection longer than 10 years who benefited from a later HAART-induced immune reconstitution (group 2).

The study was conducted with the approval of the institutional review board at the University of Illinois at Chicago. The presumptive oral lesions were diagnosed according to the criteria described by Greenspan and Greenspan[6] A normal examination was defined as a subject in whom no HIV-associated lesion was diagnosed during the clinical oral evaluation. Patients also completed a self-report questionnaire which includes demographic variables, such as age and ethnicity, as well as psychosocial variables, such as having dental insurance and receiving counseling.

Group 1 was made up of 151 individuals, with a mean age of 42.77 years (range, 18–79 years), the male/female ratio was 4.35, and the mean time since seroconversion was 62.25 months (±32.91). Group 2 was made up of 180 individuals, with a mean age of 46.71 years (range, 11–68 years), the male/female ratio was 4.55, and the mean time since seroconversion was 178.55 months (±44.81).

Forty-seven percent of the subjects in group 1 had at least one OMH with an overall average of 1.41 ± 0.55 per subjects with at least one lesion. Forty-nine percent of the subjects in group 2 had at least one OMH with an overall average of 1.58 ± 0.76 per subjects with at least one lesion. The most common lesions for both groups were OC and hairy leukoplakia which together represented more than two thirds of all diagnosed oral lesions.

To determine which variables might be confounding the relationship between normal exam and CD4 count, zero-order correlations were analyzed for the following variables: smoking, number of cigarettes smoked per day, HAART, sex, ethnicity, and dental insurance coverage. Confounding variables examined were cigarette smoking, due to the statistically significant relationship to normal examination, and HIV medication, based on theoretical rationale and previous reports associating a lower risk for OMH and HAART independently of CD4 counts.[3]

Whether a patient reported smoking cigarettes did not impact the relationship between normal examination and CD4 count or between the number of lesions a person has and CD4 count (Table 1). For group 1, the partial correlation between normal examination

and CD4 count remained significant (zero-order $r = 0.270$, $P = 0.006$; partial $r = 0.263$, $P = 0.007$) and between number of lesions a person has and CD4 count remained significant (zero-order $r = -0.286$, $P = 0.003$; partial $r = -0.28$, $P = 0.004$) when controlled for smoking. Furthermore, the relationship between number of lesions a person has and CD4 count remained significant when the reported number of cigarettes smoked per day was controlled for group 1 (zero-order $r = -.286$, $P = 0.003$; partial $r = -.29$, $P = 0.003$). Conversely, all zero-order and partial correlations for these variables were nonsignificant for group 2.

In addition to controlling for smoking behaviors, whether the person is currently on HAART was also controlled for in both groups. For group 1, the relationship between normal examination and CD4 count still approached significance after controlling for HAART (zero-order $r = 0.270$, $P = 0.006$; partial $r = 0.206$, $P = 0.061$). Although the zero-order correlation is significant and the partial correlation only approaches significance, the magnitude of these correlations supports a relationship between normal examination and CD4 otherwise not explained by whether the person is taking HAART. Again, the zero-order and partial correlations for these variables were nonsignificant for group 2.

Overall, CD4 counts are associated with oral lesions when controlling for smoking behaviors and HAART for people who contracted HIV within the last 10 years. The same pattern was not true for people who contracted HIV more than 10 years ago. However, these 2 groups did not differ on the other test variables (data not shown).

This study indicates that oral lesions remain a common morbidity in this population. Although our data, which are not longitudinal, do not allow testing whether OMHs are less frequent since the introduction of HAART, they clearly indicate that OMHs remain a common problem in HIV-infected patients in the HAART era. In our study population, close to 50% of the subjects had at least one OMH and more than a third of the subjects were diagnosed with OC, a marker of poor HIV/AIDS prognosis.[7] The predominant OMHs diagnosed in this study were OC followed by oral hairy leukoplakia, a traditional distribution which does not support a greater proportional weight for oral warts noted by some in association with HAART.[8]

Finally, this study presents evidence that the commonly described correlation between CD4 count and OMHs is not seen after more than 10 years of HIV infection. We attribute this finding to incomplete immune reconstitution associated with HAART. Then again, this finding could be an integral part of HIV natural history independently on the type of medications a patient may benefit from. Separately of this line of reasoning, we contend that although CD4 counts are merely surrogate markers for HIV disease progression, OMHs may reflect a more accurate representation of immune health. If so, this study may indicate that traditional methods assessing HIV progression and treatment response, often heavily based on stable or increasing CD4 counts, may fall short of accurately measuring immune health in patients with a history of HIV disease longer than 10 years. Future studies will address this issue by testing whether OMHs in this population is linked to disease progression and treatment failure independently of CD4 counts.

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Comparison of HIV-1 Mother-to-Child Transmission After Single-Dose Nevirapine Prophylaxis Among African Women With Subtypes A, C, and D

To the Editor:

The HIVNET 012 trial in Uganda showed that mother-to-child transmission (MTCT) of HIV-1 can be prevented by providing pregnant women and their infants with a single dose (SD) of the antiretroviral drug, nevirapine (NVP).[1,2] Safety and efficacy of 1- or 2-dose NVP prophylaxis for prevention of MTCT have been documented in other studies. We have shown that NVP resistance emerges in some women after SD NVP prophylaxis[3] and that the portion of women with NVP resistance is influenced by HIV-1 subtype.[4] At 6 to 8 weeks after SD NVP, NVP resistance was more common in women with subtype C (69.2%) than in women with subtype D (36.1%, $P < 0.0001$) or subtype A (19.4%, $P < 0.0001$).[4] Selection of NVP-resistant HIV-1 variants

TABLE 1. Risk of HIV-1 MTCT Among African Women Who Received Single-Dose Nevirapine Prophylaxis

	HIV Subtype*				OR [95% CI]		
	A (n = 158)	C (n = 452)†	D (n = 105)	R (n = 30)	C (Reference)	A	D
Infected at birth‡	8/156 (5.1%) [2.2%–9.9%]	41/452 (9.1%) [6.6%–12.1%]	9/104 (8.7%) [4.0%–15.8%]	5/30 (16.7%) [5.6%–34.7%]	1.00	0.54 [0.21–1.21]	0.95 [0.39–2.07]
Uninfected at birth but infected at 6–8 wk‡	7/145 (4.8%) [2.0%–9.7%]	22/357 (6.2%) [3.9%–9.2%]	3/93 (3.2%) [0.7%–9.1%]	1/24 (4.2%) [0.1%–21.1%]	1.00	0.77 [0.27–1.93]	0.51 [0.10–1.75]
Infected at 6–8 wk‡	15/153 (9.8%) [5.6%–15.7%]	63/398 (15.8%) [12.4%–19.8%]	12/102 (11.8%) [6.2%–19.7%]	6/29 (20.7%) [8.0%–39.7%]	1.00	0.58 [0.30–1.07]	0.71 [0.33–1.40]

*Six women had unknown HIV subtype. R indicates intersubtype recombinant.
†Subtype C includes 7 women from HIVNET 012 and 445 women from NVAZ.
‡Number of infected infants/number of infants available at each visit (% infected) [95% CI].

in women after NVP dosing could theoretically lower the efficacy of NVP prophylaxis for prevention of HIV transmission by breast-feeding in the first few weeks after birth. In the HIVNET 012 trial, most women were infected with HIV-1 subtype A or D. Risk of MTCT was slightly (but not statistically) higher in women with subtype D.[5] In this report, we combined data from the HIVNET 012 and NVAZ trials[6] to compare the risk of MTCT in women with subtype C to the risk of MTCT in women with subtypes A and D in the setting of SD NVP prophylaxis.

METHODS

Diagnosis of HIV-1 Infection in Infants

Methods used for diagnosis of HIV-1 infection in infants have been described previously for the HIVNET 012[1] and NVAZ[6] trials.

Viral Load Assays

HIV viral load testing of maternal plasma was performed using the Roche Amplicor Monitor Test Kit (Branchburg, NJ). Version 1.5 of the test kit was used in the NVAZ trial and in the HIVNET 012 trial after November 1998; before November 1998, samples in HIVNET 012 were tested with version 1.0 of the kit with additional primers.

HIV Subtyping

HIV subtyping was performed by phylogenetic analysis of *pol* region sequences, as previously described.[3] This study included 270 (88%) of the 306 Ugandan women who received SD NVP in HIVNET 012 (158 with subtype A, 7 with subtype C, and 105 with subtype D). Thirty women with intersubtype recombinant HIV-1 and 6 women with indeterminate subtypes were not included in this study. This study also included 445 Malawian women who received SD NVP in the NVAZ trial.[6] HIV-1 subtypes were obtained for 67 (15%) of those women; all had subtype C HIV-1 with no evidence of intersubtype recombination. HIV-1 subtypes were also determined for 91 women enrolled in a related study of HIV-1 MTCT performed at the same clinical site (the NVAZ late presenter trial)[7]; all of those women also had subtype C HIV-1. Identification of subtype C in all 158 of the Malawian women tested provides 95% confidence that at least 370 (98%) of the 378 additional women from NVAZ who were included in this report but who did not have subtyping performed also had subtype C. Based on these calculations, we assumed that all 445 of the Malawian women in NVAZ had subtype C infection.

Statistical Methods

Unadjusted associations of HIV-1 subtype with transmission at birth and at 6 to 8 weeks were estimated using exact tests, and adjusted associations were estimated using logistic regression. Confidence intervals (CIs) for MTCT were calculated using exact methods. The multivariate model included all 3 subtypes, with subtype C as a reference. SAS version 9.1 (SAS Institute, Inc., Cary, NC) performed these analyses.

RESULTS

We analyzed MTCT in 715 African women who received the HIVNET 012 regimen (SD NVP to the mother in labor and SD NVP to the infant shortly after birth). This cohort included 158 with subtype A, 452 with subtype C, and 105 with subtype D (see Methods). The mean $\log_{10}$ delivery viral load was similar among women with subtypes A (median, 4.28), C (median, 4.45), and D (median, 4.51; $P = 0.46$, Kruskal-Wallis test) and was similar among women in HIVNET 012 (median, 4.39, all subtypes) versus NVAZ (median, 4.46; $P = 0.23$ [Wilcoxon rank test]).

Data on MTCT were available from HIVNET 012 to infant age 18 months and from NVAZ to infant age 24 months. At 6 to 8 weeks, breast-feeding was almost universal in HIVNET 012 and NVAZ (282/283 [99.7%] and 338/341 [99.1%], respectively; $P = 0.63$ [Fisher exact test]). In contrast, at 6 months, the portion of women who were breast-feeding was significantly higher in NVAZ (299/305 [98.0%]) than in HIVNET 012 (219/258 [84.9%]; $P < 0.0001$ [Fisher exact test]). For this reason, we confined our analysis to transmission at birth and at 6 to 8 weeks of age.

In univariate analysis, there was no difference in the risk of MTCT among women with subtypes A, C, and D at birth or 6 to 8 weeks (for A vs C vs D: for infants infected at birth, $P = 0.28$; for infants uninfected at birth but infected at 6–8 weeks, $P = 0.58$; and for infants infected at 6–8 weeks, $P = 0.16$ [Fisher exact test]; Table 1). In a multivariate model including $\log_{10}$ HIV-1 maternal viral load at delivery, age, parity, and HIV-1 subtype, MTCT was associated with maternal viral load for infants infected at birth (odds ratio [OR], 2.01; 95% CI, 1.36–2.96; $P = 0.0004$), for infants uninfected

at birth but infected at 6 to 8 weeks (OR, 2.49; 95% CI, 1.53–4.04; $P = 0.0002$), and for infants infected at 6 to 8 weeks (OR, 2.01; 95% CI, 1.46–2.78; $P < 0.0001$), but was not associated with age, parity, or HIV-1 subtype.

DISCUSSION

This report extends our analysis of MTCT and NVP resistance in women enrolled in the HIVNET 012 and NVAZ trials. In a previous report, we demonstrated that women with subtype C HIV-1 were more likely to have NVP-resistant HIV-1 variants detected 6 to 8 weeks after SD NVP than women with either subtype A or D.[4] In this report, we find no significant difference in the risk of MTCT among women with these 3 subtypes. This provides empiric evidence that selection of NVP-resistant HIV-1 variants in women after SD NVP dosing does not significantly increase the risk of MTCT during the first few weeks of life. However, even in this large study of 715 women, a difference in MTCT risk may not be apparent because of low transmission rates in the setting of SD NVP prophylaxis. Based on the CIs for the ORs comparing MTCT in the 3 subtypes, we cannot exclude the possibility that subtypes A and D, which have less resistance, also have as much as a 50% lower risk of MTCT than subtype C.

Other smaller studies have examined the association of *env*, *gag*, or LTR subtypes with MTCT in the absence of antiretroviral prophylaxis. Among 28 women from Tanzania, there was no significant difference in MTCT risk among women with *env* and LTR subtypes A, C, and D or intersubtype recombinant HIV-1.[8] In contrast, another study of 51 matched transmitting and nontransmitting women from Tanzania found a higher risk of MTCT in women with *gag* and *env* regions that were either both subtype C or recombinant (different combinations of subtypes A, C, and D), compared with women with *gag* and *env* regions that were both subtype D.[9] Among 253 Tanzanian infants, a higher rate of in utero HIV-1 infection was observed with *env* subtype C compared with *env* subtype A or D.[10] In another study of 414 Kenyan women, there was a higher risk of MTCT for women with *gag* and *env* regions that were either both subtype D or recombinant (D/A or A/D) compared with women with *gag* and *env* regions that were both subtype A.[11]

In this study, HIV-1 subtype was determined using HIV-1 *pol* (protease and reverse transcriptase) sequences. This region of the HIV-1 genome is the most relevant to use of antiretroviral drugs for prevention of MTCT. We recently observed an association between HIV-1 *pol* region replication capacity and MTCT among women with subtype C,[12] suggesting that *pol* region determinants may influence transmission risk. Our inability to detect a difference in the risk of MTCT among women with *pol* region subtypes A, C, and D who received SD NVP prophylaxis may be influenced by several factors. First, the power to see differences in transmission among different subtypes may be reduced because the number of transmission events is reduced by NVP prophylaxis. Second, the SD NVP regimen could also lower MTCT to a greater degree in women with certain subtypes (eg, reflecting subtype-based differences in drug susceptibility). Finally, in countries such as Uganda where multiple HIV-1 subtypes cocirculate, the subtype of different regions of the viral genome may be different because of intersubtype recombination.[13] Therefore, it is possible that associations between HIV-1 subtype and MTCT risk might be observed in women in HIVNET 012 and NVAZ, if subtyping were based on a different region of the HIV-1 genome. Further studies are needed to identify HIV-1 determinants for MTCT and to examine the association of HIV-1 subtype and MTCT in the presence and absence of antiretroviral prophylaxis.

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ERRATA

In the article by Spacek et al. appearing in the *Journal of Acquired Immune Deficiency Syndromes*, Vol. 41, No. 5, pp. 607–610, entitled "Evaluation of a Low-Cost Method, the Guava EasyCD4 Assay, to Enumerate CD4-Positive Lymphocyte Counts in HIV-Infected Patients in the United States and Uganda," the list of authors was incorrect. It should have included Moses Kamya, MBChB, MMed, MPH (affiliated with Makerere University, Kampala, Uganda), as the sixth author in the list, to read:

> "Lisa A. Spacek, MD, PhD, Hasan M. Shihab, MBcHB, Fred Lutwama, MBcHB, Jean Summerton, BA, Harriet Mayanja, MD, Moses Kamya, MBChB, MMed, MPH, Allan Ronald, MD, Joseph B. Margolick, MD, PhD, Tricia L. Nilles, BS, and Thomas C. Quinn, MD"

"Nonnegligible Increasing Temporal Trends in Unprotected Anal Intercourse Among Men Who Have Sexual Relations With Other Men in Montreal" by Clemon George, PhD, Michel Alary, MD, PhD, Joanne Otis, PhD, Eric Demers, MSc, Benoît Mâsse, PhD, René Lavoie, MSc, Robert S. Remis, MPH, MD, Bruno Turmel, MD, Jean Vincelette, MD, Raymond Parent, MSc, Roger LeClerc, and the Omega Study Group, Omega Cohort, Montreal, Québec, Canada, which appeared in the *Journal of Acquired Immune Deficiency Syndromes*, volume 42, number 2, pages 207–212, is a duplication of the article that appeared in the *Journal of Acquired Immune Deficiency Syndromes*, volume 41, number 3, pages 365–370. When citing, please use the volume 41, number 3 reference.

We apologize to the authors of the article and the readers of *JAIDS* for this error.