

Relationship between Adherence Level, Type of the Antiretroviral Regimen, and Plasma HIV Type 1 RNA Viral Load: A Prospective Cohort Study

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Abstract

The relationship between adherence, antiretroviral regimen, and viral load (VL) suppression was assessed through a 1 year prospective follow-up study among 1142 HIV-infected patient. Patients on antiretroviral therapy who attended to the pharmacy during a 6-month period were considered eligible. Those included in the final analysis were patients who had been taking the same antiretroviral therapy for ≥ 6 months since their inclusion. The cohort included patients taking first line therapy ($n = 243$) and antiretroviral-experienced patients ($n = 899$). Naive patients who were included had to have reached undetectable VL at enrollment. Antiretroviral-experienced patients with detectable VL determinations in the previous 6 months were excluded. Adherence was measured by means of announced pill counts and dispensation pharmacy records. Of patients, 58% were taking NNRTI, 31.4% boosted PI, and 10.6% unboosted PI-based regimens. Overall, the relative risk of virologic failure was 9.0 (95% CI 4.0–20.1) in patients with adherence 80–89.9%, 45.6 (95% CI 19.9–104.5) with adherence 70–79.9%, and 77.3 (95% CI 34.2–174.9) with adherence $< 70\%$, compared with adherence of $\geq 90\%$. The risk of virologic failure in patients with adherence $< 90\%$ taking unboosted PI was 2.5 times higher than the group taking boosted PI (95% CI 1.2–5.3). There were no statistical differences in patients taking boosted PI and those who were taking NNRTI. Less than 95% of adherence is associated with high virologic success. For patients taking NNRTI- or boosted PI-based regimens with adherence rates of 80%, the failure rate is $< 10\%$. These data do not affect the goal of achieving the highest level of adherence possible.

Introduction

THE INTRODUCTION of highly active antiretroviral therapy (HAART) in 1996 changed the clinical course of HIV, with a significant decline in morbidity and mortality.¹ Nowadays, HIV is a chronic disease, so patients have to take antiretroviral therapy for a long time with substantial adverse effects and sometimes with complex regimens. Evidence suggests that HIV-positive patients have problems taking antiretroviral medication correctly^{2,3} and nonadherence has been recognized as one of the main causes of treatment failure. Studies have also demonstrated that incomplete adherence to HAART is associated with an increase in morbidity⁴ and, even, in mortality.⁵ It has been well recognized that antiretroviral efficacy depends on adherence. However, it is still not clear how much adherence is enough.^{6–11} Paterson *et al.*¹⁰ reported 95% of adherence to therapy as the minimum level necessary to maintain viral

load suppression. However, patients included in this study were taking regimens based in unboosted protease inhibitors. Lately, other authors have reported higher viral suppression rates with adherence rates not as perfect as 95% in patients treated with more modern regimens.^{9,12}

Unfortunately, taking more than 95% of the prescribed regimen is a difficult goal to achieve and maintain. Consequently it is extremely important to know the adherence cut-off values that can lead to viral failure.

In this study we investigated the adherence level to obtain viral load suppression with the current regimens.

Materials and Methods

The study was conducted in the Outpatients Pharmacy Department of an HIV/AIDS reference institution located in Barcelona. All HIV-infected patients on antiretroviral therapy who attended to the pharmacy to pick up medication

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during a 6-month period (from October 1, 2004 to April 1, 2005) were considered eligible for this study. Patients taking first line therapy and antiretroviral-experienced patients were included. Naive patients who were included had to have reached undetectable viral load at enrollment. Antiretroviral-experienced patients with undetectable viral load at enrollment and with no detectable determinations in the previous 6 months were included in order to avoid the bias caused by virologic failure due to resistance. Patients included in the final analysis were those who had been taking the same antiretroviral therapy for more than 6 months since the date of inclusion. Patients taking combinations that included only nucleoside reverse-transcriptase inhibitors (NRTIs) were excluded.

We collected the following baseline data: age, sex, risk factors for HIV infection, clinical data, and medication used.

Viral load and adherence were measured in each visit (usually every 4 months).

Plasma HIV-RNA copy levels were evaluated using the commercially available AMPLICOR quantitative restriction transcriptase polymerase chain reaction assay (Roche Diagnostic Systems, Branchburg, NJ) according to the manufacturer's instructions. The lower limit of detection of the assay was 200 copies/ml. Samples with less than 200 copies/ml were retested using the Ultra Direct Assay (Roche Molecular Systems, Alameda, CA) with a lower limit of quantification of 50 copies/ml.

Virologic failure was defined as two consecutive HIV RNA levels greater than 200 copies/ml.

Adherence was measured in each visit by means of announced pill counts or dispensation pharmacy records (when pill counts were not possible).

Our institution has developed a program to provide patient information about antiretroviral therapy in order to improve adherence and decrease drug errors. In this program announced pill counts are routinely used to measure adherence. Every patient initiating antiretroviral therapy is interviewed by a pharmacist after the medical visit. During this interview the pharmacist explain to the patient about the therapy and edits an individualized information leaflet. Written information includes a daily medication schedule and some information on general considerations and adverse drug reactions for every drug. After the initial meeting adherence assessments are performed after each medical visit. Medical visits usually take place every 4 months. However, visits can take place every month if the patient has medical problems or every 6 months in a few special cases. Patients have to bring their remaining pills to the pharmacy in each visit so that the pharmacist could calculate adherence. The number of missed doses is computed from the difference between the actual and expected number of pills. Nevertheless, if patients forget to bring their pills we use dispensation pharmacy records. Approximately 70% of the measures were calculated by means of pill counts and 30% by dispensation pharmacy records. There are many methods to measure adherence each with its own advantages and disadvantages. Because no perfect method has been identified, the recommendation is to use several measures in an attempt to address methodologic difficulties. We chose these two methods to measure adherence because both of them are practical,

inexpensive, and sufficiently accurate to be used in a clinical routine.

Patients were classified based on defined adherence levels of <70%, 70–79.9%, 80–89.9%, and $\geq 90\%$. The often cited Paterson study categorized adherence as <70%, 70–79.9%, 80–89.9%, 90–94.9%, and $\geq 95\%$. We chose to combine the last two categories as $\geq 90\%$ because we found no difference between 90–94.9% and $\geq 95\%$.

The antiretroviral regimens were classified as combinations based on nonnucleoside reverse transcriptase inhibitors (NNRTI), boosted protease inhibitors (PI), and unboosted PI.

The duration of follow-up was 1 year. For those patients who discontinued therapy before 1 year, the follow-up period was time until discontinuation (always more than 6 months).

Statistical analyses

Descriptive analysis was based on percentage and frequency for qualitative variables and standard deviation (SD) or median and interquartile range (IQR) for quantitative variables. Chi-square or Fischer's exact test was used for analyzing contingency tables. Quantitative characteristics were compared between groups using the Mann-Whitney or Kruskal-Wallis test. The correlation between two quantitative variables was analyzed by means of Spearman's coefficient (ρ). Odds ratios (OR) and 95% confidence intervals (95% CI) of having a detectable viral load were estimated for treatment groups (PI, NNRTI, relative to boosted PI) and for adherence levels (<70, 70–79.9, 80–89.9, relative to ≥ 90). Multinomial logistic regression was estimated in order to determine the relative risk ratio of the adherence level (≥ 90 level of reference) for treatments groups (boosted PI reference group). All tests were two sided with a confidence level set to 95%.

The analysis was performed using STATA (StataCorp. 2005. Stata Statistical Software: Release 9.2. Stata Corporation, College Station, TX).

Results

Descriptive characteristics

Initially 2011 eligible patients were included; however, 413 antiretroviral-experienced patients were excluded because they had detectable viral load (VL) determinations in the previous 6 months, 208 patients were lost of follow-up, 202 additional patients were excluded because they stopped or changed their antiretroviral combination before 6 months of follow-up, and 46 patients were excluded because they were taking combinations that included only NRTI. Finally, 1142 patients were included in this study.

The descriptive characteristics at baseline were the following: 864 (75.6%) were men; the median age was 44 years old; the route of infection was homosexual in 454 (39.8%), heterosexual in 334 (29.2%), and intravenous drug use (IVDU) in 275 (24.1%). At enrollment into the study the mean CD4 cell count was 564.9 cells/mm³ (SD 285.8).

Only 243 (21.3%) were naive. Most patients had received various combinations. They had been under antiretroviral treatment for a mean of 85.6 months (SD 40.5). They had been receiving the latest combination for a mean of 32.4 months

(SD 20.6). The most frequent regimens used were combinations based on NNRTI in 662 patients (58%), followed by boosted PI in 359 (31.4%), and finally unboosted PI in 121 (10.6%). Table 1 shows the baseline characteristics of the 1142 patients included, patients taking first line therapy and antiretroviral-experienced patients.

Variables associated with virologic failure

Over the follow-up period, mean adherence was 95.7% for those with an undetectable VL (1059 patients) and 76.3% for those with a detectable VL (83 patients) ($p < 0.005$).

Variables included in the analysis were sex, age, risk factor, CD4 lymphocyte count at enrollment, duration of antiretroviral therapy, duration of current antiretroviral regimen, and type of antiretroviral regimen received. However, after adjustment by logistic regression, statistically significant variables associated with virologic failure were adherence rates and antiretroviral regimen. There were no differences between the first-line and antiretroviral-experienced patients.

Figure 1 represents the relationship between adherence and virologic failure in patients taking NNRTI, boosted PI, and unboosted PI.

Overall, the relative risk of virologic failure was 9.0 (95% CI 4.0–20.1) in patients with adherence 80–89.9%, 45.6 (95% CI 19.9–104.5) in patients with adherence 70–79.9%, and 77.3 (95% CI 34.2–174.9) in patients with adherence less than 70% compared with patients with adherence of 90% or greater.

In patients taking unboosted PI the relative risk of virologic failure was 26.2 (95% CI 3.0–230.7) in patients with adherence 80–89.9% and 207.5 (95% CI 16.0–2696.2) in patients with adherence 70–79.9% compared with patients with adherence of 90% or greater. There were no patients with adherence less than 70% and controlled viral load.

In patients taking boosted PI the relative risk of virologic failure was 21.5 (95% CI 2.6–174.4) in patients with adherence 80–89.9%, 124.7 (95% CI 15.1–1032.9) in patients with adherence 70–79.9%, and 212.0 (95% CI 26.4–1702.4) in patients with adherence less than 70% compared with patients with adherence of 90% or greater.

In patients taking NNRTI the relative risk of virologic failure was 4.4 (95% CI 1.4–13.3) in patients with adherence 80–89.9%, 22.4 (95% CI 7.5–66.8) in patients with adherence 70–79.9%, and 36.9 (95% CI 12.7–107.5) in patients with adherence less than 70%.

Logistic regression analyses also showed that the risk of virologic failure in patients with an adherence rate of <90 taking unboosted PI was 2.5 times higher compared to the group taking boosted PI (95% CI 1.179–5.341). There were no statistical differences in patients taking boosted PI and those who were taking NNRTI.

Variables associated with adherence

Variables identified as predictors of compliance adherence were type of antiretroviral regimen, total number of pills to be taken per day, and the number of daily doses.

Adherence depends on the regimen class. Patients who were taking NNRTI-based therapies presented better adherence levels [96.2 (90–100)] than patients taking PI-based therapies [92.6 (84.6–97.9)] ($p < 0.000$).

Spearman’s correlation coefficient showed the relationship between adherence and the number of pills to be taken per day. As the number of pills increases the patient is less likely to take the medications as prescribed ($\rho = -0.2$; $p < 0.001$).

No statistically significant differences in adherence were found between once daily and twice daily, but both of them had better adherence rates than three or more times a day: 94.59 (88.02–99.18) vs. 91.19 (81.04–95.41) ($p < 0.000$).

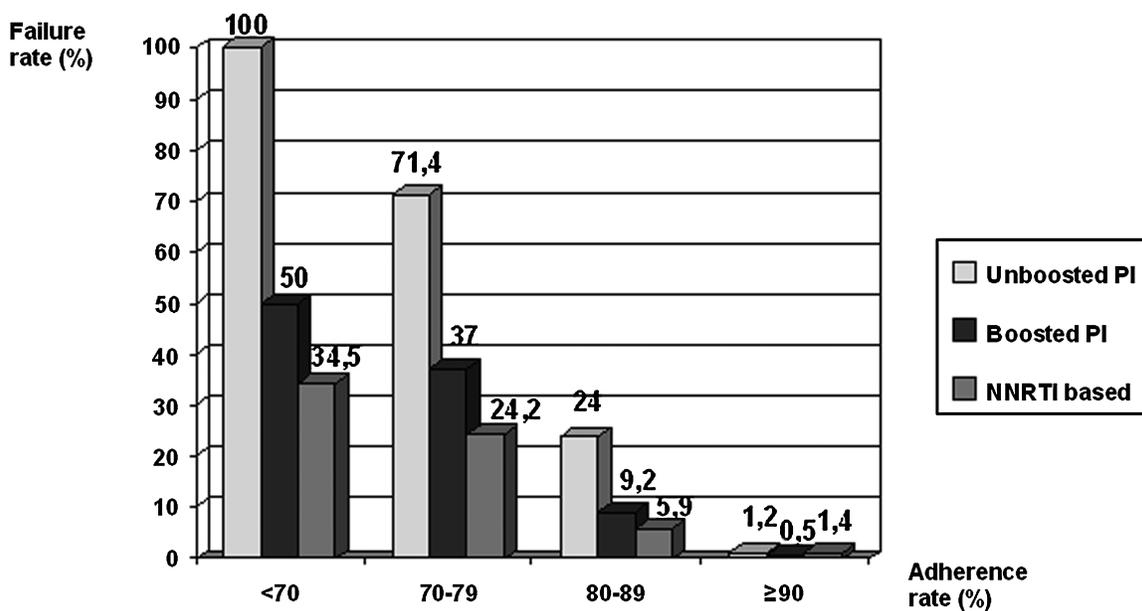


FIG. 1. Adherence to antiretroviral therapy and virologic failure.

TABLE 1. BASELINE CHARACTERISTICS OF THE 1142 PATIENTS INCLUDED

	<i>All patients</i>	<i>First line therapy</i>	<i>HAART experienced</i>
Number of patients included	1142	243 (21.3%)	899 (78.7%)
Lost to follow-up	208 (18.2%)	49 (20.2%)	159 (17.7%)
Male	864 (75.6%)	191 (78.6%)	669 (74.4%)
Age, mean years \pm SD	44.0 \pm 9.6	44.1 \pm 9.6	43.8 \pm 9.6
Risk factor			
Injection drug use	275 (24.1%)	41 (16.9%)	234 (26.0)
Heterosexual	334 (29.2%)	77 (31.7%)	257 (28.6%)
Homosexual	454 (39.8%)	112 (46.1%)	342 (38.0%)
Other	79 (6.9%)	13 (5.3)	66 (7.3%)
CD4 lymphocyte count (cells/mm ³)	564.9 \pm 285.8	527.5 \pm 281.9	569.2 \pm 292.2
Duration of HAART, mean months \pm SD	85.6 \pm 40.5	32.5 \pm 23.7	89.4 \pm 41.9
Duration of current HAART regimen, mean months \pm SD	32.4 \pm 20.6	32.5 \pm 23.7	30.5 \pm 20.1
Type of HAART received			
Based on NNRTI	662 (58.0%)	184 (75.7%)	478 (53.2%)
Nevirapine	342	56	286
Efavirenz	320	128	192
Based on boosted PI	359 (31.4%)	42 (17.3%)	317 (35.3%)
Lopinavir	204	37	167
Atazanavir	108	2	106
Saquinavir	15	1	14
Fosamprenavir	14	2	12
Indinavir	10	0	10
Tipranavir	8	0	8
Based on unboosted PI	121 (10.6%)	17 (7.0%)	104 (11.6%)
Atazanavir	56	5	51
Nelfinavir	50	8	42
Saquinavir	8	1	7
Indinavir	7	3	4

Discussion

The adherence rate was higher than expected. One possible reason is the measurement bias. It is difficult to assess medication compliance because of methodologic difficulties in measuring drug adherence.

As previously explained, we chose announced pill counts and pharmacy records to measure adherence, because both of them are practical, inexpensive, and provide quantitative data.¹³ However, both can overestimate adherence when they are compared with MEMS.¹⁴ Data from patients with low adherence are reliable but it is not possible to ensure that patients with perfect pill counts are taking the medication. In spite of this, the pill counts method has been used in clinical practice and in adherence research.¹⁵ Although our data are measured by a combination of pill counts and pharmacy records, we believe that when taken together with the size of our sample, the data provide strong evidence. Moreover, adherence overestimation should be equivalent in patients with controlled and uncontrolled viral load.

The second possible reason for the high adherence rate is that patients were included only if they had been taking the same antiretroviral combination for 6 months and had no detectable viral load determinations in the previous 6 months. Therefore, we excluded many nonadherent pa-

tients, patients with voluntary interruptions, and patients lost to follow-up.

There are several limitations to our study. Because this is an unblinded study, patients may be inclined to dump pills, leading to social desirability bias. This bias should be comparable in both groups of patients.

Other limitation to the study that deserve to be mentioned is that differences in patient characteristics can affect antiretroviral pharmacokinetics. However, we assumed that adherence was the most important factor in determining overall drug exposure.

It is well recognized that antiretroviral efficacy depends on adherence. Nevertheless, it is still not clear how much adherence is enough. The study conducted by Paterson *et al.*¹⁰ showed that patients with 95% or greater adherence had a superior virologic outcome, a greater increase in CD4 lymphocyte count, and a lower hospitalization rate than did patients with lower levels of adherence. Similarly, Low-Beer *et al.*¹⁶ reported that $\geq 95\%$ adherence was associated with high virologic success and that success rates drop off sharply with decreasing levels of adherence to therapy. However, patients included in these studies were taking PI regimens without boosting with ritonavir.

Lately, other authors have reported higher viral suppression with adherence rates not as high as 95%. Bangsberg¹² reported that viral suppression is common with a 54–100%

mean adherence level when patients are taking NNRTI regimens. In the same way data from Maggiolo *et al.*⁹ showed that patients who were receiving PI regimens and who had an adherence rate of up to 85% had a virologic failure rate of >20%; however, in patients who were receiving NNRTI and who had an adherence rate of $\leq 75\%$, the virologic failure rate was >10%.

Our results are highly consistent with those found by Maggiolo *et al.*⁹ and Bangsberg *et al.*,¹² but this is the first study in which data on NNRTI, boosted PI, and unboosted PI are analyzed individually. The results of this study confirm that NNRTI- and boosted PI-based regimens lead to VL suppression with less adherence rates than the older PI-based regimens without boosting.

The risk of virologic failure was not equally distributed between the diverse types of regimens. The risk of virologic failure in nonadherent patients was significantly higher than in those who were taking unboosted PI regimens, compared to the groups taking boosted PI or NNRTI. Patients who were taking an unboosted PI-based regimen with an adherence rate between 80 and 89.9% presented a failure rate of 24%; however, those who were taking boosted PI or NNRTI with the same adherence level presented failure rates in only less than 10%. There were no statistical differences in patients taking boosted PI and those who were taking NNRTI.

Summarizing, moderate levels of adherence led to higher rates of viral suppression in NNRTI or boosted PI-treated patients compared to patients treated with unboosted PI. In this study we did not measure resistance but it is important to take into account the results published by other authors. Bangsberg *et al.*⁸ reported that while use of an NNRTI can lead to a greater proportion of individuals with viral suppression, the majority of NNRTI-treated individuals with levels of adherence too low for viral suppression developed resistance. In contrast, few individuals on a single PI therapy with low to moderate levels of adherence developed PI resistance. It is apparent that patients with low levels of adherence to NNRTI therapy are at a high risk for resistance, creating a precarious balance between viral suppression and drug resistance.

Regimen class was also associated with adherence. Patients who were taking NNRTI-based therapies presented better adherence levels than patients taking PI-based therapies. It could be due to the fact that patients in treatment with PI therapies were taking more pills daily than patients taking NNRTI therapies. Another cause could be that PI regimens usually have more adverse effects than NNRTI regimens and can influence adherence.¹⁷

Other parameters significantly correlated with adherence rate were total number of pills to be taken per day and the number of daily doses. As the number of pills increases the patient is less likely to take the medications as prescribed. However, we did not find a specific number that would tip the balance toward nonadherence. With fewer pills, adherence is higher and more homogeneous and with more pills adherence is lower and more erratic. With reference to the number of daily doses, no statistically significant differences in adherence were found between once daily and twice daily, but both of them had better adherence rates than three or more times a day. It is still not clear if once vs. twice has any advantages. Some studies have found sta-

tistically significant differences^{9,18} between them but not others.¹⁹ Moreover, patients who do not take one dose in the one daily dose regimen may be at higher risk for low drug levels than those who take treatment two or more times a day. Patients prefer once daily doses²⁰ but the benefit is not clear.

Unfortunately, for many patients adherence can be difficult. HIV-positive patients have to take antiretroviral therapy for a long time with considerable adverse effects and sometimes with complex regimens. It is extremely important to evaluate adherence regularly, as well as to assess potential barriers that can affect long-term adherence. Barriers can be grouped into three categories: related to the patient (stress, depression, drug or alcohol addiction, lack of support from family and friends, social stigma, and so on), the health care system (relationship between patient and provider, availability, and so on), and the regimen (complexity, adverse effects and so on).²¹ Because there are many factors that can affect adherence, it is necessary to obtain the participation of a multidisciplinary care team of to ensure long-term adherence.

In summary, our data show that virologic success is possible with less than 95% adherence. The adherence cutoff value depends on the type of HAART. For patients taking NNRTI- or boosted PI-based regimens with adherence rates of 80%, the failure rate is less than 10%. These data do not affect the goal of achieving the highest level of adherence possible.

Disclosure Statement

No competing financial interests exist.

References

1. Palella FJ, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, *et al.*: Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV outpatient study investigators. *N Engl J Med* 1998;338:853–860.
2. Gordillo V, del Amo J, Soriano V, and González-Lahoz J. Sociodemographic and psychologic variables influencing adherence to antiretroviral therapy. *AIDS* 1999;13:1763–1769.
3. Knobel H, Alonso J, Casado JL, Collazos J, Gonzalez J, Ruiz I, *et al.*: Validation of a simplified medication adherence questionnaire in a large cohort of HIV-infected patients: The GEEMA Study. *AIDS* 2002;16(4):605–613.
4. Bangsberg DR, Perry S, Charlebois ED, Clark RA, Roberston M, Zolopa AR, *et al.*: Non-adherence to highly active antiretroviral therapy predicts progression to AIDS. *AIDS* 2001;15:1181–1183.
5. De Olalla G, Knobel H, Carmona A, Guelar A, Lopez-Colomes JL, and Cayla JA: Impact of adherence and highly active antiretroviral therapy on survival in HIV-infected patients. *J Acquir Immune Defic Syndr* 2002;30:105–110.
6. Gross R, Bilker WB, Friedman HM, and Strom BL: Effect of adherence to newly initiated antiretroviral therapy on plasma viral load. *AIDS* 2001;15:2109–2117.
7. Townsend ML, Jackson GL, Smith R, and Wilson KH: Association between pharmacy medication refill-based adherence rates and CD4 count and viral load responses: A retrospective analysis in treatment-experienced adults with HIV. *Clin Ther* 2007;29:711–716.

8. Bangsberg D, Acosta E, Gupta R, Guzman D, Riley E, Harrigan P, *et al.*: Adherence-resistance relationships for protease and non-nucleoside reverse transcriptase inhibitors explained by virological fitness. *AIDS* 2006;20:223–231.
9. Maggiolo F, Ravasio L, Ripamonti D, Gregis G, Quinzan G, Arici C, *et al.*: Similar adherence rates favor different virologic outcomes for patients treated with nonnucleoside analogues or protease inhibitors. *Clin Infect Dis* 2005; 40(1):158–163.
10. Paterson DL, Swindells S, Mohr J, Brester M, Vergis EN, Squier C, *et al.*: Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. *Ann Intern Med* 2000;133:21–30.
11. Shuter J, Sarlo JA, Kanmaz TJ, Rode RA, and Zingman BS: HIV infected patients receiving lopinavir/ritonavir-based antiretroviral therapy achieve high rates of virologic suppression despite adherence rates less than 95%. *J Acquir Immune Defic Syndr* 2007;45:4–8.
12. Bangsberg D: Less than 95% adherence to nonnucleoside reverse-transcriptase inhibitor therapy can lead to viral suppression. *Clin Infect Dis* 2006;43:939–941.
13. Codina C, Martínez M, Tuset M, del Cacho E, Martín MT, Miró JM, *et al.*: Comparison of three methods to calculate adherence in patients receiving antiretroviral treatment. *Enferm Infecc Microbiol Clin* 2002;20(10):484–490.
14. Liu H, Golin CE, Miller LG, Hays RD, Beck CK, Sanandaji S, *et al.*: A comparison study of multiple measures of adherence to HIV protease inhibitors. *Ann Intern Med* 2001;134:968–977.
15. Miller LG and Hays RD: Measuring adherence to antiretroviral medications in clinical trials. *HIV Clin Trials* 2000;1:36–46.
16. Low-Beer S, Yip B, O'Shaughnessy MV, Hogg RS, Montaner JS, *et al.*: Adherence to triple therapy and viral load response. *J Acquir Immune Defic Syndr* 2000;23:360–361.
17. Martín MT, del Cacho E, López E, Codina C, Tuset M, de Lazzari E, Miró JM, Gatell JM, and Ribas J: Adverse side effects of antiretroviral therapy: Relationship between patients' perception and adherence. *Med Clin (Barc)* 2007; 129(4):127–133.
18. Portsmouth S, Osorio J, McCormick K, Gazzard B, and Moyle G: Better maintained adherence on switching from twice-daily to once-daily therapy for HIV: A 24-week randomized trial of treatment simplification using stavudine prolonged-release capsules. *HIV Med* 2005; 6(3):185–190.
19. Claxton AJ, Cramer J, and Pierce C: A systematic review of the associations between dose regimens and medication compliance. *Clin Ther* 2001;23:1296–1310.
20. Moyle G: The assessing patients' preferred treatments (APPT-1) study. *Int J STD AIDS* 2003;14(S1):34–36.
21. Knobel H, Escobar I, Polo R, Ortega L, Martín-Conde MT, Casado JL, *et al.*: Recommendations from GESIDA/SEFH/PNS to improve adherence to antiviral treatment (2004). *Enferm Infecc Microbiol Clin* 2005;23(4):221–231.

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