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EVOLUTION OF HIV-1 VIRAL LOAD IN PATIENTS FOLLOWED-UP FOR OVER 3 YEARS

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ABSTRACT
Objectives: To describe the evolution of a Human Immunodeficiency Virus Type 1 (HIV-1) infected patient cohort monitored for over 1,000 days.
Methods: HIV-1 Viral Load (VL), CD4/l lymphocyte values and antiretroviral therapies given to the patients were evaluated throughout the follow-up period. We present a retrospective descriptive study of the HIV-1 VL determinations performed on 369 individuals followed-up for over 1,000 days.
Results: The "non-detectable" VL (< 400 RNA copies/ml) percentage increased inversely with the decrease in VL above the detection limit (> 100.000 copies/ml) from the interval of 0-75 days up to the interval of 501-1,000 days (t-test, p=0.005); at that point, results switched to the opposite.
Conclusions: Both CD4/ cell count lower than 200x106 and patients receiving highly active antiretroviral therapies (HAART) were related to "non-detectable" VL levels. In our series the time period between 700 and 1,000 days can be the maximum interval for benefits from therapy and virology evaluation.

Key words: HIV, Viral Load, Follow-up, HAART, observational study.

RESUMEN
Objetivo: Describir la evolución de una cohorte de pacientes con infección por el Virus de la

Inmunodeficiencia Humana (VIH) monitorizados durante más 3 años.
Métodos: Durante el período de seguimiento se han evaluado en 396 individuos con infección VIH, seguidos durante más de 1000 días los parámetros de carga viral, valores de linfocitos CD4 y terapia antirretroviral.
Resultados: Las porcentajes de carga viral no detectable (<400 copias/ARN/ml) se incrementaron de manera proporcional a cómo descendieron los valores de carga viral elevada (>100.000 copias/ARN/ml), y su rango adquirió significación desde el intervalo de 0-75 días de seguimiento al de 501-100 días (t-test, p=0.005). Los recuentos de CD4 bajos (<200) en pacientes que recibieron Terapia antirretroviral de alta eficacia se asociaron a valores indetectables de carga viral.
Conclusiones: En nuestra serie el período situado entre 700-100 días representó el intervalo de máximo beneficio para la evaluación virológica y terapéutica.

Palabras clave:HIV, Carga viral, Seguimiento, HAART, estudio observacional

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INTRODUCTION

The appearance of highly active antiretroviral therapies (HAART) and the patients Human Immunodeficiency Virus Type 1 (HIV-1) viremia evaluation by plasma analysis have brought about an important change in clinical attention to HIV-1 patients. HIV-1 infection has become chronic and almost asymptomatic, with mortality reduced between 50-90% in patients with high treatment adherence [1,2]. However, the introduction of these antiretroviral therapies has not totally eradicated the virus from the organism [3]. Clinical follow-up of HIV-1 patients has lengthened, bringing new implications: treatment failure, documented mutations involving therapy resistance and possible treatment alternatives. These circumstances have also increased interest in describing real behavior in large patient samples followed-up in health centers [4-5]. This has lead in turn to the increase of studies on variability in clinical practices [6-11] in the search for helpful data.

The objective of this study was to evaluate HIV-1 VL evolution, CD4 /cell count and antiretroviral treatments of patients in clinical practice during long-term follow-up.

MATERIALS AND METHODS

The Microbiology Laboratory of the University Teaching Hospital of Valladolid (Spain) analyzes plasma viremia for eight hospitals, three prisons and other health centers treating HIV patients (centers for intravenous drug addicts individuals, out-patient clinics, etc.) in the Autonomous Community of "Castilla y León".

This study used samples from all these patients based on the existence of a follow-up period above but close to 1,000 days (average length 1,125 days; interval 1,001-1,326). This criterion yielded 369 individuals followed periods of time from September 1996 to June 2000. The individuals were 74% (273 subjects) male and 60.4% (223 patients) older than 30, based on information available. A HIV infection risk factor was found in 70.5% (N=260) of the individuals; the most frequent modality was addiction through intravenous drugs (65.4%, N=170), followed by sexual transmission (20.8%, N=54) and blood derivative transfusions (3.8%, N=10), while only three patients had mothers with HIV antibodies. The risk factor was not noted in the case files of the remaining 8.8% of the patients. Based on cohort cases with available data, HIV infection was principally diagnosed between 1990 and 1995 (122 individuals); 70 individuals were diagnosed before 1990 and 63 after 1995. No HIV diagnosis date was available for 121 patients (32.8%).

All samples used for HIV-1 RNA analysis were plasma samples. The Microbiology Laboratory used the standard and ultrasensitive versions of the Cobas Amplicor® HIV-1 MonitorTM technique (Roche Diagnostics, Branchburg, NJ, USA) and the QuantiplexTM® HIV-1 RNA 3.0 assay (Bayer Corporation Diagnostics Division, Tarrytown, NY, USA) throughout the study period. For this study, due to the time span covered, viremia analysis results lower than 400 copies RNA/ml have been considered as "non-detectable" regardless of the threshold level of the technique used. All VL above 100,000 copies RNA/ml (the limit of the Cobas Amplicor® HIV-1 MonitorTM Test) were considered as "above 100,000 copies."

The length of patient follow-up was divided into irregular intervals to make analysis easier. VL, CD4/l lymphocyte count and antiretroviral therapy were determined at the moment of initiating follow-up and also in time intervals of 0-75 days, 76-105, 106-200, 200-500, 501-1,000 days and over 1,000 days. The

average number of VL determination per patient performed during the study period was 9.5.

The antiretroviral therapy that the patients received was indicated by a variable having two categories: Treatments with a single drug or with a combination of two drugs were considered "pre-HAART therapies"; the use of three or more drugs was denominated "HAART therapy."

Patient virological, immunological and therapeutical situations were described with varied categories and percentage calculations. The relation between VL and the immunological and therapeutical parameters was calculated by statistical significance, using the SPSS 9.0 computer program.

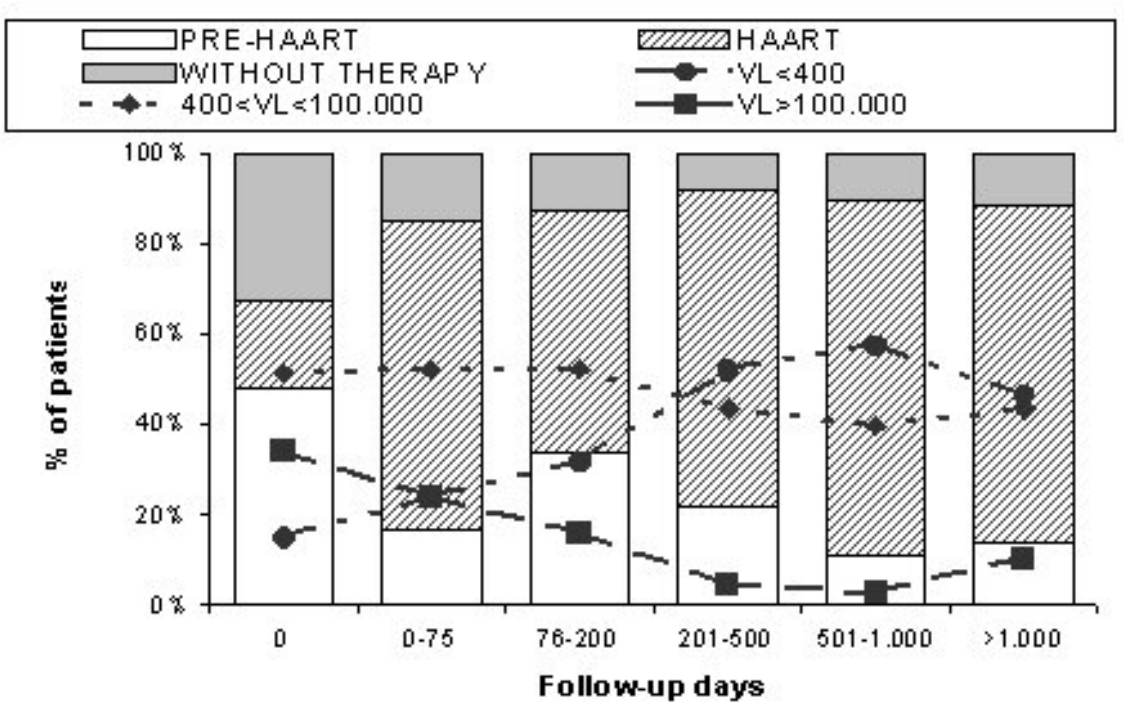
RESULTS

Viral Loads lower than threshold level were presented by 14.9% of the patients (N=55) at the beginning of follow-up. At that moment 33.9% had VL > 100,000 copies RNA/ml and the remaining 51.2% patients had VL between 400 and 100,000 copies RNA/ml. Patient VL in our sample showed steady intermediate VL levels during the first six months (51.2%, 52.2% and 52.2% up to the interval 76-200 days), with a drop after that moment (43.5%, 39.8% and 43.3%). "Non-detectable" VL increased inversely to the decrease in VL above detection limit after the interval 0-75 days; at the time interval 501-1,000 days this tendency reversed, VL above 100,000 copies RNA/ml increasing (from 2.7% to 10.4%) while "non-detectable" VL decreased (57.5% to 46.6%).

Immunological state at the moment of incorporation into the study was registered for 320 subjects (86.7%); 110 patients (34.4%) presented CD4/l lymphocyte counts lower than 200 x106 and 65 (20.3%) counts higher than 500 x 106. CD4/l lymphocyte counts remained practically stable throughout the follow-up period, with percentages about 65% for counts above 200 x106 and about 35% for those below that.

Figure 1 shows the evolution of VL and CD4/l lymphocyte counts throughout follow-up. Low VL (VL < 400 copies RNA/ml) is associated with CD4/l lymphocyte counts above 200 x106 throughout follow-up (between 73.1% and 80.2% of the subjects with "non-detectable" VL present lymphocyte CD4/mm3 counts below 200 x106). Differences in CD4/l lymphocyte count distribution are statistically significant only for follow-up above 1,000 days (p=0.005).

Figure 1. Evolution of HIV Viral Load percent (lines) and of CD4/l lymphocyte counts (bars).

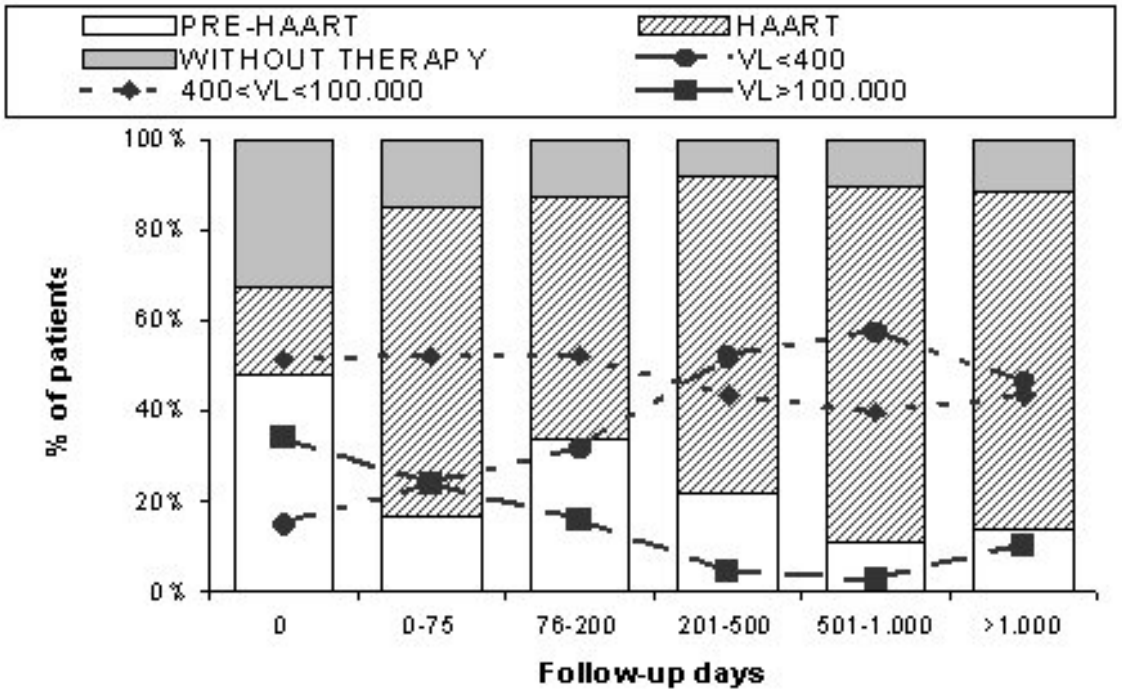


Cohort patients for whom data on antiretroviral therapy was obtained at the beginning of follow-up (N=302, 81.8%) were divided into monotherapy regimes (27, 10.2%), bitherapy (101, 38%) or triple therapy (87, 19.2%); the remaining 87 individuals (32.7%) were not receiving any antiretroviral therapy when they were included in our study. HAART therapy individuals generally increased throughout follow-up: 19.2%, 68.5%,

53.9%, 70.1%, 78.9% and 74.5%. At the end of the study 292 individuals (79.1%) had received highly active antiretroviral therapy (HAART).

HAART therapy was more frequent in patients with "non-detectable" VL than in those undergoing single or double-drug therapy (grouped together as "pre-HAART") or those without antiretroviral therapy. The lowest VL percent was found in those not receiving any antirretroviral therapy. Differences were statistically significant for follow-up intervals above 1,000 days (p=0.004) and the interval 201-500 days (p=0.001). Figure 2 shows VL progression and patient treatments.

Figure 2. Evolution of patient HIV Viral Load percent (lines) and antiretroviral therapy (bars).



During the study period a single drug was used for 106 patients (28.7%). The rest of the patients received the following therapies: 84 subjects (22.8%) had 2 different drug therapies, 76 (20.6%) had 3 changes of therapy, 47 patients (12.7%) had 4 changes, 12 (3.3%) had 5, and 3 patients (0.8%) received 6 and 7 changes in therapy respectively. To establish the average number of treatments among the patients for whom this information was available, the coefficient between the number of treatments and the individuals included in them was calculated; 789 treatments yielded a coefficient of 2.4 treatments per patient during follow-up.

DISCUSSION

The patients from this cohort were similar with respect to availability of resources and potential access to the antiretrovirals used. The patients began their follow-up toward the end of 1996 and early 1997; the only therapy available at that time was the pre-HAART modality (single and double-drug). These patients were subject to changes in therapy based on recommendations from experts arising throughout the follow-up period [12-16] (principally switches to HAART therapy). The incorporation of most of the individuals included in HAART modalities occurred in the follow-up interval of 0-75 days. This was also the period when the proportion of patients having VL over 100,000 copies RNA/ml decreased, while the proportion of those with VL under 400 copies RNA/ml increased simultaneously. These two VL categories varied in that time period, with no modification observed in the VL interval of 400-100,000 copies RNA/ml. These results might be considered unique to our cohort, but the study by Lepri et al, 2001 [17], evaluating highly active therapies in the context of a clinical trial, shows similar changes.

Later controls in our study included more and more patients receiving HAART therapy. A simultaneous increase in the proportion of plasma viremia below threshold level can be seen, along with decreases in the other two VL study levels. Several publications consider six months to be the maximum time interval in which highly active therapy should be evaluated [7,16], so our study has assimilated this 6-month control within the time interval of 201-500 days follow-up. The elevated number of patients who still have VL levels above detection limit at this follow-up moment is noteworthy in our results. One explanation could be the

fact that different therapy modalities began sequentially in our series, so this length of follow-up (201-500 days) is not long enough to evaluate therapy success. However, it is evident that all subjects included in the time cut of 201-500 days have a previous control at least six months earlier (even though the follow-up period of some of them is longer than six months).

Our study results show an unfavorable VL evolution at the end of patient follow-up. It is important to note that this VL increase does not correspond to modifications in the distribution of therapy modality proportions given with respect to previous controls. Consideration of therapies available when current therapy has failed [9,15,19] and the appearance of antiretroviral-resistant HIV strains [20,21] are two aspects widely discussed in literature published. For our patients, the maximum time period of benefit from therapies and virology monitoring was between 700 and 1,000 days

We studied the number of antiretroviral regimes each patient received throughout their follow-up. Conscious of the limitations of the study, we can say that our patients received an average of approximately 2.4 different therapies. Despite the fact that we did not investigate therapy change causes, it is valid to say that this average implies a different therapy regime every four VL controls. Palella et al, 2002 [22] had described a different evolution with the second or more HAART therapy respect the first one.

In our opinion, providing real findings about the patient progression in health centers can be very helpful. It would be useful to know the experiences of other groups working in situations similar to ours.

REFERENCES

1.- Palella FJ Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, Aschman DJ, Holmberg SD. Declining morbidity and mortality among patients with advanced Human Immunodeficiency Virus infection. N Engl J Med 1998;338:853-860.

2.- Paterson DL, Swindells S, Mohr J, Brester M, Vergis EN, Squier C, Wagener MM, Singh N. Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. Ann Intern Med 2000;133:21-30.

3.- Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson RE, Quinn TC, Chadwick K, Margolick J, Brookmeyer R, Gallant J, Markowitz M, Ho DD, Richman DD, Siliciano RF. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science 1997;278:1295-1300.

4.- Miller V, Sabin CA, Phillips AN, Rottmann C, Rabenau H, Weidmann E, Rickerts V, Findhammer S, Helm EB, Staszewski S. The impact of protease inhibitor-containing highly active antiretroviral therapy on progression of HIV disease and its relationship to CD4 and viral load. AIDS 2000;14:2129-2136.

5.- Hubert JB, Burgard M, Dussaix E, Tamalet C, Deveau C, Le Chenadec J, Chaix ML, Marchadier E, Vilde JL, Delfraissy JF, Meyer L, Rouzioux. Natural history of serum HIV-1 RNA in 330 patients with a known date of infection. AIDS 2000;14:123-131.

6.- Del Amo J, Del Romero J, Barrasa A, Pérez-Hoyos S, Rodriguez C, Diez M, García S, Soriano V, Castilla J, and the Grupo de Seroconvertores de la Comunidad de Madrid. Factors influencing HIV progression in a seroconverter cohort in Madrid from 1985 to 1999. Sex Transm Inf 2002;78:255-260.

7.- Grabar S, Pradier C, Le Corfec E, Lancar R, Allavena C, Bentata M, Berlureau P, Dupont C, Fabbro-Peray P, Poizot-Martin I, Costagliola D. Factors associated with clinical and virological failure in patients receiving a triple therapy including a protease inhibitor. AIDS 2000;14:141-149.

8.- Howard AA, Arnsten JH, Lo Y, Vlahov D, Rich JD, Schuman P, Stone VE, Smith DK, Schoenbaum EE; the HER Study Group. A Prospective study of adherence and viral load in a large multi-center cohort of HIV-infected women. AIDS 2002;16:2175-2182.

9.- Mocroft A, Devereux H, Kinloch-de-Loes S, Wilson D, Madge S, Youle M, Tyrer M, Loveday C,

Phillips AN, Johnson MA. Immunological, virological and clinical response to highly active antiretroviral therapy treatment regimens in a complete clinic population. AIDS 2000;14:1545-1552.

10 .- Phair JP, Mellors JW, Detels R, Margolick JB, Munoz A. Virologic and immunologic values allowing safe deferral of antirretroviral therapy. AIDS 2002;16:2455-2459.

11. Welch K, Morse A, Clark R, Ogbuokiri T. Factors associated with incomplete virological response to highly active antiretroviral therapy. Clin Infect Dis 2000;30:407-408.

12.- BHIVA Guidelines Co-ordinating Committee. British HIV Association guidelines for antiretroviral treatment of HIV seropositive individuals. Lancet 1997;349:1086-1092.

13.- Carpenter CC, Fischl MA, Hammer SM, Hirsch MS, Jacobsen DM, Katzenstein DA, Montaner JS, Richman DD, Saag MS, Schooley RT, Thompson MA, Vella S, Yeni PG, Volberding PA. Antiretroviral therapy for HIV infection in 1996. Recommendations of an International panel. International AIDS Society- USA. JAMA 1996;276:146-154.

14.- Carpenter CCJ, Cooper DA, Fischil MA, Gatell JM, Gazzard BG, Hammer SM. Antiretroviral therapy in adults. Updated recommendations of an International panel. International AIDS Society-USA panel. JAMA 2000;283:381-391.

15.- Miro JM, Antela A, Arrizabalaga J, Clotet B, Gatell JM, Guerra L, Iribarren JA, Laguna F, Moreno S, Parras F, Rubio R, Santamaria JM, Viciana P. Recommendation of GESIDA (AIDS Study Group)/National Plan on AIDS with respect to the anti-retroviral treatment in adult patients infected with the human immunodeficiency virus in the year 2000 (I). Enferm Infec Microbiol Clin 2000;18:329-351.

16.- Moreno S, Arrizabalaga J, Gatell JM, Clotet B, Aguirrebengoa K, Antela A, Iribarren JA, Laguna F, Miro JM, Ocana I, Rubio R, Viciana P, Podzamczer D. Recommendations on antiretroviral treatment. The AIDS Study Group of the Spanish Society of Infectious Diseases and Clinical Microbiology. Med Clin (Barc) 1998;110:109-106.

17.- Lepri AC, Miller V, Phillips AN, Ravenau H, Sabin C, Staszewski S. The virological response to highly active antiretroviral therapy over the first 24 weeks of therapy according to the pre-therapy viral load and the weeks 4-8 viral load. AIDS 2001;15:47-54.

18.- Mocroft A, Phillips AN, Miller V, Gatell J, van Lunzen J, Parkin JM, Weber R, Roge B, Lazzarin A, Lundgren JD; EuroSIDA study group.The use of and response to second-line protease inhibitor regimens: results from the EuroSIDA study. AIDS 2001;26:201-209.

19.- Panel on Clinical Practices for Treatment of HIV Infection convened by the Department of Health and Human Services (DHHS) and the Henry J. Kaiser Family Foundation. Guidelines for the use of antirretroviral agents in HIV-infected adults and adolescents, February, 2002. www.aids.nih.gov.

20.- Lorenzi P, Opravil M, Hirschel B, Chave JP, Furrer HJ, Sax H, Perneger TV, Perrin L, Kaiser L, Yerly S. Impact of drug resistance mutations on virologic response to salvage therapy. AIDS 1999;13:F17-F21.

21.- Parkin NT, Deeks SG, Wrin MT, Yap J, Grant RM, Lee KH, Heeren D, Hellmanna NS, Petropoulos CJ. Loss of antiretroviral drug susceptibility at low viral load during early virological failure in treatment-experienced patients. AIDS 2000;14:2877-2887.

22.- Palella Jr FJ, Chmiel JS, Moorman AC, Holmberg SD: The Outpatient Study Investigators. Durability and predictors of success of highly active antiretroviral therapy for ambulatory HIV-infected patients. AIDS 2002;16:1617-1626.

Comment of the reviewer Suthon Vongsheree MD. HIV/AIDS Laboratory, Thai NIH. Department of Medical Science. Ministry of Public Health, Thailand.

The manuscript describes chronologically change of HIV-1 and CD4 cell levels among 369 ARV treated patients with a long follow-up period over 3 years. Successful treatment was observed during 201-1000 days of treatments monitored by 9.5 VL tests/individuals, retrospectively.

Given to the comprehensive data of this article, the manuscript should be accepted for publication.

Comment of the reviewer Angel San Miguel, MD. PhD. Clinical Chemistry Service. "Del Rio Hortega" Universitary Hospital. Valladolid, Spain.

En este trabajo los autores estudian y describen la evolución de los niveles de carga viral en una cohorte de 369 pacientes infectados con VIH-1 y monitorizados durante 1000 días de tratamiento. Los parámetros utilizados en el estudio son, carga viral de VIH-1, valores de los niveles de lintocitos CD4, y los intervalos de seguimiento de las terapias antiretrovirales.

En los resultados obtienen que le porcentaje de carga viral "no detectable" (< 400 copias RNA/ml) aumenta de forma inversamente proporcional a la disminución en el límite de detección (> 100.000 copias RNA/mL) en el intervalo de 0-75 días de tratamiento, hasta el intervalo de 501-1000 días de tratamiento que ocurre lo contrario.

Las conclusiones más relevantes del estudio, según los autores son: (1) que de los parámetros estudiados, los niveles de CD4 < 200 x 106 y los pacientes que reciben terapias antiretrovirales altamente activas, han podido ser relacionados con niveles de carga viral de VIH-1 "no detectable"; y (2) que el intervalo de tiempo entre 700 y 1000 días puede ser el intervalo máximo para los beneficios de la terapia y de la evaluación virológica del paciente.

Mi juicio personal sobre el trabajo es ampliamente favorable: está bien diseñado, su modelo experimental es adecuado, los datos parecen fidedignos y las conclusiones aportan resultados deseables. Por ello, me parece obligado recomendar su publicación en Electronic Journal of Biomedicine. Como sugerencia, quisiera animar a los autores, a los cuales aprecio y respeto, a que en esta misma revista presenten los resultados de las resistencias de VIH a los antiretrovirales (bien sobre el grupo de pacientes estudiado u otro).

Comment of the reviewer María Luisa Ávila Agüero MD. Jefa del Servicio de Infectología. Hospital Nacional de Niños Dr. Carlos Sáenz Herrera. San José, Costa Rica.

He revisado el artículo y lo encuentro muy adecuado para publicación, no tendría que hacersele ninguna corrección.

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