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ORIGINAL ARTICLE

Effects of first-line anti-retroviral therapy on blood coagulation parameters of HIV-infected patients attending a tertiary hospital at Abuja, Nigeria

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Abstract

Background: Blood coagulation abnormalities are common in persons infected with the human immunodeficiency virus (HIV). However, few studies showed the association of these abnormalities with anti-retroviral therapy (ART). *Objective:* This cross-sectional study investigated the effects of ART on blood coagulation parameters of patients infected with HIV attending HIV special clinics of the University of Abuja Teaching Hospital (UATH), Gwagwalada, Abuja, Nigeria. *Material and Methods:* A total of 191 patients comprising 128 HIV subjects on ART (test subjects) and 63 other HIV patients not on ART (control subjects) were included in the study. $CD4^+$ lymphocyte counts, platelet counts, prothrombin time (PT) and partial thromboplastin time with kaolin (PTTK) of subjects were determined using flow cytometry, automated hematology analyser and Quick one-stage methods respectively. *Results:* Of the total test subjects, 21 (16.4%) were CD4 lymphopaenic, and the mean $CD4^+$ cell count for the test subjects was statistically higher than that of the control subjects (578 versus 322 cells/ mm^3) ($p = 0.014$). Eight (6.3%) of test subjects had prolong PTTK, and the mean values of PT and PTTK were statistically not significant between test subjects and control subjects ($p = 0.358$ and $p= 0.141$ respectively). Eight (6.3%) of test subjects had thrombocytopaenia, the mean platelet count was significantly lower than that of the control subjects (238 versus 278.6 x 10^9/L, $p = 0.001$), and also varied significantly with the duration of ART ($p = 0.0086$). *Conclusion:* Findings from this study revealed ART decreased platelet counts of HIV-infected individuals, but did not affect the PT and PTTK results.

Keywords: Anti-retroviral therapy; HIV; platelet counts; coagulation assay; haemostasis

INTRODUCTION

The Global prevalence of HIV is on the increase because people on anti-retroviral therapy (ART) are living longer, although new infections decreased from 3.3 million in 2002, to 2.3 million in 2012.[1] In 2005, an estimate of 2.3 million AIDS-related deaths was reported, making it the highest global mortality figure. However, this decreased to 1·6 million by 2012. An estimated 9·7 million people in low- and middle-income countries were started on ART by 2012. New insights into the mechanisms of latent infection and the importance of reservoirs of HIV infections might eventually lead to a cure.[1]

Several studies reported that risk of thrombosis increases in association with progressed stages of HIV infection.[2] Acute inflammation due to HIV infection is one of the important causes

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of coagulation disorders.[3] Cytokines act as mediators for activation of the coagulation system with more effect on the extrinsic pathway than the intrinsic.[3] Involved cytokines include tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and IL-6.[4] Vascular endothelial cells interacting with cytokines released from leukocyte, adhesion molecules and growth factors play important roles in up-regulation of the coagulation system.[4]

Vascular endothelium and liver are important components of the coagulation system.[5] HIV infection leads to endothelial dysfunction; so activation and consumption of coagulation factors occurs, these result to coagulopathy.[6] Impairment in liver function during HIV infection by reducing coagulation factors adds to the compromised coagulation state.[7] Since ART is known to induce hepatotoxicity, coagulation (especially Vitamin-K dependent) factors are also affected and this ultimately leads to impaired synthesis of these factors.[7]

The primary tests routinely used for the assessment of coagulopathy are prothrombin time (PT) and partial prothrombin time (PTTK). Additionally, platelets have significant role in haemostasis. Owing to cytokines and platelet activation factor (PAF) secreted during HIV infection, platelet dysfunction in association with impaired thrombopoiesis lead to thrombocytopaenia.[8]

Although ART has decreased the mortality of HIV infection, increased non-AIDS related conditions such as cardiovascular diseases and coagulopathies have became serious co-morbidities.[8] Adverse effects of ART vary from drug to drug, ethnic differences, individual differences, and due to interaction with other non-ART drugs. Hypersensitivity to some drugs may also occur in some individuals. Several studies also showed that some coagulation parameters exhibit considerable variations at different periods of life and disease conditions.[9] Although ART has dramatically improved the survival in HIV-infected patients, precautions still need to be taken to prevent ART-related haematoxicity and other life threatening side effects.[7,9] Owing to the fact that there are scanty available clinical safety data on the use of first-line ART with the combination of Lamivudine, Zidovudine, and Nevirapine in Nigerian HIV-infected patients, there is a need to examine the potential coagulation dysfunctions that could be associated with this regimen.

In view of these, the study sought to determine the effect of ART on subjects' platelet counts, evaluate the extrinsic and intrinsic coagulation integrity of HIV-infected subjects on ART and compare it with that of HIV seropositive ART-naïve subjects.

MATERIALS AND METHODS

Study design

This was a cross-sectional study conducted from March to August 2015. It included a control group (HIV seropositive subjects not on ART drugs) and test group (apparently healthy HIV seropositive subjects on ART drugs). They were screened and confirmed HIV seropositive using Uni-Gold Recombigen (Trinity Biotech, Wicklow, Ireland) and Determine™ (Alere Medical Co., Chiba, Japan) test strips, both of which are HIV 1/2 rapid diagnostic kits. According to the data provided by the test manufacturers, Determine™ has sensitivity and specificity of 99.91% and 98.16% respectively with serum and plasma, while Unigold™ has 100% for whole blood/ plasma/serum.

Study area

Abuja is the capital city of Nigeria. It is located in the center (9°4′0″N 7°29′0″E) of Nigeria, within the federal capital territory (FCT). At the 2006 census, the city had a population of 776,298, making it one of the top ten most populous cities in Nigeria. The FCT Abuja has six area councils (Gwagwalada included). According to demographic data, the population of Abuja's Urban Area as of 2012 was 2,245,000, making it the fourth largest urban area in Nigeria. This was a hospital-based study carried out at a tertiary hospital, the University of Abuja Teaching Hospital (UATH) located in Gwagwalada area council. It is a center for President's Emergency Plan for AIDS Relief (PEPFAR) interventions; a non-governmental global granted cohort program aimed at providing relief to HIV-infected patients. The center provides diagnosis for new cases of HIV infections and monitors those on therapy. Subjects for this study were recruited from this center. All laboratory parameters were analyzed at the haematology laboratory of UATH, Nigeria.

Sample size calculation

Prior to this study, there has never been a similar study in Nigeria, hence, the sample size was determined using the 2012 national HIV

prevalence rate, 3.4% (National Agency for the Control of AIDS). Therefore, the minimum sample size at 95% confidence level was 51. In order to boost the statistical credence of the study, the sample size was increased to 128 subjects who consented to participate.

Study population

A total of one hundred and ninety-one (191) subjects were enrolled into the study. One hundred and twenty-eight (128) of these served as the test subjects who were known HIV seropositive and on ART medication at HIV PEPFAR Clinic of the UATH. The remaining sixty three (63) served as control subjects who were HIV seropositive, but not on any non-ART medication (i.e. ART naïve). The selection process of appropriate subjects was done by HIV physicians and nurses of the HIV PEPFAR Clinic of UATH by purposive sampling method. All recruited subjects consented voluntarily for the study.

Criteria for subjects' selection

Inclusion criteria for test subjects: Those diagnosed as HIV seropositive, but seronegative for hepatitis B and C viruses, and apparently healthy as screened by HIV physicians. They were not on any non-ART medication for the past 30 days.

Exclusion criteria for test subjects: Those with known history of blood coagulation / bleeding disorders and not on any non-ART medication for the last 30 days. Patients with any condition other than HIV that could cause bone marrow suppression or thrombocytopaenia were excluded.

Inclusion criteria for control subjects: Those confirmed ART naïve HIV patients, but seronegative for hepatitis B and C viruses, and apparently healthy individuals who were not on any non-ART medication for the last 30 days. Those with known history of blood coagulation or bleeding disorders were excluded.

Sample collection and processing

Five ml of venous blood was collected individually from every participant using new sterile syringes. Two (2) ml was carefully dispensed into ethylene diamine tetraamine acid (EDTA) container for $CD4^+$ cell count while the remaining blood was filled to the 'mark' noted on the 3.8% sodium-citrate tubes for the PT and PTTK tests. These tubes were then appropriately labelled with participants' study number. Plasmas from these sodium citrate tubes were separated after centrifugation at 2500 rpm for 10 minutes. The EDTA anticoagulated whole blood was analysed for $CD4^+$ cell count and haematology (specifically, platelet count), while the sodium citrated anticoagulated blood was analysed for PT and PTTK. All tests were performed within 4 hours of blood sample collection.

Laboratory analytical methods

An automated haematology analyser was used to estimate the platelets of the studied subjects, while for their PT and PTTK Quick one-stage method was used based on the Clinical and Laboratory Standard Institute (CLSI) alongside kit manufacturer's instructions for the tests procedure.[1,2] The PTTK measures the clotting time of plasma in the presence of contact factors and indicates the overall efficiency and integrity of the intrinsic coagulation pathway, while the PT measures the clotting time of plasma in the presence of tissue factors and so indicates the overall efficiency of the extrinsic pathway.

Determination of $CD4^+$ cell count

Cluster of Differentiation-4^+ ($CD4^+$) cell count in whole blood was determined using Partec Cyflow ® analyser model SL3 based on manufacturer's instruction. It works on the principle of light scatter (due to different size or granularity of the cells) combined with fluorescence of cells after staining with monoclonal antibodies to cell surface markers tagged to fluorescent dye. Cyflow reagents and consumables were used according to the manufacturer's instructions. Twenty (20) µL of EDTA anticoagulated blood was pipetted into a Partec test tube. Ten µL of CD4-phycoeryrthrin conjugated monoclonal antibody supplied by Partec was added to the tube containing the blood and the reactants and then incubated for 15 minutes at room temperature in the dark. Following incubation, 800 µL of no lyse buffer, supplied by Partec was added to the tube and the resultant solution was gently vortex-mixed. The tube was then plugged into the Cyflow counter for automatic counting.

The histogram and absolute counts were displayed and printed automatically. The histogram showed direct counting result in terms of absolute $CD4^+$ T-lymphocytes/µL. The $CD4^+$ T-lymphocytes with high fluorescence appear in a prominent peak at the right of the histogram,

whereas the weaker but also $CD4^+$ monocytes appear to the left without any overlap with $CD4^+$ T-lymphocytes. $CD4^+$ ($CD3^+CD4^+CD8^-$) cells were gated (regions drawn on fluorescence scatter plots and histograms to selectively focus on populations of interest) by the process of `primary CD4 gating'. Absolute $CD4^+$ cell counts were then determined using single-platform methodology. Internal quality control for pipetting errors was based on CD3 replicates using the Immunocount II quality control programme.

Ethical approval, informed consent and questionnaire

This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the ethical research committee of the University of Abuja Teaching Hospital (Approval number: FCT/UATH/HREC/PR/347). The study was appropriately explained to all participants. More so, they individually gave verbal and/or written consent for inclusion before being recruited for the study. All data were analysed anonymously throughout the study. Structured questionnaires were used to obtain data such as age, gender, type of ART and duration of treatment for test subjects.

Statistical Analysis

Data generated from the investigation was systematically analysed. Data were presented as mean ± standard deviation (SD). Chi-square test for independence was used to determine relationship. Student's t- test for unpaired data and one-way ANOVA with Bonferroni post-test analyses were used to compare variables. Statistical Package for Social Sciences (SPSS) software version 20 (California Inc., USA) was used for all the analyses. A two sided $p < 0.05$ at 95% confidence interval (CI) was considered statistically significant.

RESULTS

The median age of test subjects was 35.7 years. Those aged >40 years constituted the majority of the subjects with 39.1% (50) while those <18 years and those between 19-30 years constituted the least number of study subjects with 16 (12.5%) each.

Out of the 128 test subjects, 21 were CD4 lymphopaenic (<250/mm^3) (Table 1). The $CD4^+$ cell count of the test subjects was not significantly associated with their platelet counts (p = 0.440) (Table 2). The mean $CD4^+$ cell count for the test subjects was statistically higher when compared with that of the control subjects [578 versus 322 cells/mm^3 (p = 0.014) (Table 3)].

Only 8 (6.3%) of test subjects had prolonged PTTK. The mean value of prothrombin time was not significantly higher in the test subjects (p = 0.358) when compared with that of the control subjects (Table 3). The mean value of PTTK was not significantly higher in the test subjects when compared with that of the control subjects (p = 0.1407) (Table 4). The prevalence of thrombocytopaenia in test subjects was 6.3%. The mean value of platelet count among the test subjects was significantly lower (p = 0.0010) when compared with that of the control subjects (238 versus 278.6 x 10^9/L) (Table 4). There was a significant decrease in the platelet counts of the HIV patients on ART over time with increased duration of ART as compared to the control group (Table 4).

DISCUSSION

Thrombocytopaenia has been known to be a common hematological disorder in persons infected with HIV. Although often asymptomatic,

TABLE 1: Relationship between anti-retroviral therapy status and $CD4^+$ immunity in HIV-infected subjects

$CD4^+$ count	ART Status		Total (%)
	HIV Positive on ART	HIV Positive off ART	
Low (%)	21 (11.0)	28 (14.7)	49 (25.7)
Normal (%)	107 (56.0)	35 (18.3)	142 (74.3)
Total	128 (67.0)	63 (33.0)	191 (100)

Note: Low CD4 count = < 250 cells /mm^3; normal CD4 count = ≥250 cells/mm^3

TABLE 2: Relationship between platelet counts and CD4⁺ cell count of HIV patients on ART

CD4⁺ count	Platelet counts		
	Low (%)	Normal (%)	Total (%)
Low	1 (4.8)	20 (95.2)	21 (100)
Normal	6 (5.6)	101 (94.4)	107 (100)
Total	7 (5.4)	121 (94.5)	128 (100)

χ^2 = 0.1362, df=1, *p*= 0.7121, OR=0.8417 (95% CI: 0.09598 – 7.380)
Note: Low platelet count = < 150 cells/mm^3; normal platelet count = 150 - 450 cells/mm^3

TABLE 3: Comparison of clotting profiles and CD4⁺ cell counts of test and control subjects

	Mean ± SD		p-value
	Test subjects (n=128)	Control subjects (n=63)	
PT (Sec)	13.04 ± 1.80	12.81 ± 1.21	0.3580
PTTK (Sec)	28.52 ± 7.57	27.02 ± 3.61	0.1407
Platelets (x10^9/L)	238.00 ± 74.12	278.6 ± 86.34	**0.0010***
CD4⁺ cell count (cells/mm^3)	578.3 ± 78.5	322.5 ± 62.4	**0.014***

*Significant difference as determined by unpaired Student's t-test.
PT=Prothrombin Time, PTTK=Partial Thromplastin Time with Kaolin.

TABLE 4: Effects of duration of ART on clotting profile of test and test subjects

	Duration of ART (years) Mean ± SD				p-value
	0-5 (n=72)	6-11 (n=51)	≥12 (n=5)	Control (n=63)	
PT (Sec)	13.03 ± 1.64	13.16 ± 2.05	12.00 ± 0.71	12.81 ± 1.21	0.3690
PTTK (Sec)	29.49 ± 8.35	27.57 ± 6.38	24.20 ± 4.71	27.02 ± 3.61	0.0720
Platelets (x10^9/L)	240.89 ± 83.50	231.69 ± 60.70	261.40 ± 57.03	278.60 ± 86.34	**0.0086*#**

*Significant difference between control subjects and 0-5 duration group by Bonferroni post-test.
#significant difference between and control subjects and 6-11 duration group by Bonferroni post-test.

low platelet counts in HIV patients undergoing ART may be associated with a variety of haemostatic abnormalities. Despite the high prevalence of HIV and wide accessibility of ART in Nigeria, there is paucity of information with regards to the effects of ART on platelet counts and routine blood coagulation parameters of HIV patients.

The findings from this study show significant increase of platelet counts in patients on ART than ART-naive patients and that this increase was independent of the increase in CD4⁺ cell count of the patients on ART. These findings are not in consonant with other studies which reported relative increase in platelet counts in HIV-infected patients with thrombocytopaenia who were treated with zidovudine,[10-12] but these responses were not sustained after follow-up

tests. Our findings are consistent with that of Bouldouyre *et al*[13] and Vannappagari *et al*[14] who reported that ART significantly caused thrombocytopaenia in HIV-infected patients.

We recorded a 6.3% prevalence of thrombocytopaenia in HIV patients on ART. This value is slightly lower than that of the South Korean study by Choi *et al*[12] who reported a 7% thrombocytopaenia, and Firnhaber *et al*[15] who also demonstrated a 7% thrombocytopaenia from their studies. The relatively slightly low prevalence from our study could be explained by the following reasons: the defining value for thrombocytopaenia in our study was higher than those in previous studies, and the exclusion of patients with secondary causes of cytopaenia from our subjects.

In our study, CD4 lymphopaenia was defined by $CD4^+$ cell count < 250 cells/mm^3. Low $CD4^+$ cell count and concurrent ART could be risk factors for thrombocytopaenia, as bone marrow suppression by ART and blood cells production defects caused by HIV infection are responsible for the thrombocytopaenia.[16-19] It is well known that the incidence and severity of thrombocytopaenia are generally correlated with the stage of HIV infection,[20,21] as reflected by significant progression of thrombocytopaenia with an increase in ART duration in our test subjects.

Some retrospective studies have suggested that ART, particularly protease inhibitors play a role in the increased incidence of thrombosis seen in HIV-infected patients.[22] Another study reported rapid progress of Kaposi sarcoma due to zidovudine induced thrombocytopaenia.[23] Therefore close clinical examinations need to be done on HIV patients on ART particularly those with other risks of developing coagulopathy.

Only a limited number of studies described the effect of ART on coagulation markers. Findings from this study revealed that ART was not significantly associated with PT and PTTK ($p>0.05$). This finding did not corroborate with previous studies which indicated persistent haemostatic abnormalities in HIV patients on ART.[24,25] However, reports by Fezoui *et al*[26] did not show significant effects of ART on blood coagulation parameters. The main reasons for this disparity could be attributable to differences in study location/race or due our inability to conduct platelet factor and endothelial function assays.

Despite the coagulopathy in some test subjects, $CD4^+$ cell counts of HIV subjects on ART was significantly higher than HIV subjects not on ART. This is a clear indication of the beneficial role of ART in boosting the cellular immunity of HIV infected individuals, as they prevent/ minimise further destruction of $CD4^+$ cells by the virus.

Conclusion

Thrombocytopaenia is a common feature among HIV-positive patients. However, there are few reports about this condition after the ART era. Findings from this study revealed that ART significantly decreased platelet counts of HIV infected patients, but did not affect the PT and PTTK results.

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Conflict of interest: Authors declare that there is no conflict of interest associated with this manuscript. *Authors' contribution:* Idris Abdullahi Nasir and Adebola Owolagba conceptualised the study design and conducted the study as well as developed the initial draft manuscript. Abdurrahman Elfulaty Ahmad and Muhammad Maimadu Barma gave input into the design and statistical aspects of the study, and critically revised the manuscript before publication. Peter Omale Musa, Dele Ohinoyi Amadu and Yakubu Ibrahim provided guidance on study design and development of the study. All authors read and approved the final manuscript.

REFERENCES

1. Maartens G, Celum C, Lewin SR. HIV infection: epidemiology, pathogenesis, treatment and prevention. Lancet. 2014; 384: 258-71.
2. Lijfering WM, Ten Kate MK, Sprenger HG, van der Meer J. Absolute risk of venous and arterial thrombosis in HIV-infected patients and effects of combination antiretroviral therapy. J Thromb Haemost. 2006; 4: 1928-30.
3. Levi M, van der Poll T, ten Cate H, van Deventer SJ. The cytokine-mediated imbalance between coagulant and anticoagulant mechanisms in sepsis and endotoxaemia. Eur J Clin Invest. 1997; 27: 3-9.
4. Hack CE. Tissue factor pathway of coagulation in sepsis. Crit Care Med. 2000; 28: S25-30.
5. Karpatkin S, Nardi M, Green D. Platelet and coagulation defects associated with HIV-I-infection. Thromb Haemost. 2002; 88: 389-401.
6. Andrade ACO, Cotter BR. Endothelial function and cardiovascular disease in HIV infected patient. Braz J Infect Dis. 2006; 10: 139–45.
7. Oguntibeju OO, van den Heever WMJ, Van Schalkwyk FE. Effect of a liquid nutritional supplement on viral load and haematological parameters in HIV-positive/AIDS patients. Br J Biomed Sci. 2006; 63: 134–9

8. Levi M, Keller TT, van Gorp E, ten Cate H. Infection and inflammation and the coagulation system. Cardiovasc Res. 2003; 60: 26-39.
9. Kibaru EG, Nduati R, Wamalwa D, Kariuki N. Impact of highly active antiretroviral therapy on hematological indices among HIV-1 infected children at Kenyatta National Hospital-Kenya: retrospective study. AIDS Res Ther. 2015; 12: 26.
10. Glatt AE, Anand A. Thrombocytopenia in patients infected with human immunodeficiency virus: treatment update. Clin Infect Dis. 1995; 21: 415-23.
11. Ballem PJ, Belzberg A, Devine DV, *et al*. Kinetic studies of the mechanism of thrombocytopenia in patients with human immunodeficiency virus infection. N Engl J Med. 1992; 327: 1779-84.
12. Choi SY, Kim I, Kim NJ, *et al*. Hematological manifestations of human immunodeficiency virus infection and the effect of highly active antiretroviral therapy on cytopenia. Korean J Hematol. 2011; 46: 253-7.
13. Bouldouyre MA, Charreau I, Marchou B, *et al*. Incidence and risk factors of thrombocytopenia in patients receiving intermittent antiretroviral therapy: a substudy of the ANRS 106-window trial. J Acquir Immune Defic Syndr. 2009; 52: 531-7.
14. Vannappagari V, Nkhoma ET, Atashili J, Laurent SS, Zhao H. Prevalence, severity, and duration of thrombocytopenia among HIV patients in the era of highly active antiretroviral therapy. Platelets. 2011; 22: 611-8.
15. Firnhaber C, Smeaton L, Saukila N, *et al*. Comparisons of anemia, thrombocytopenia, and neutropenia at initiation of HIV antiretroviral therapy in Africa, Asia, and the Americas. Int J Infect Dis. 2010; 14: e1088-92.
16. Rarick MU, Espina B, Montgomery T, Easley A, Allen J, Levine AM. The long-term use of zidovudine in patients with severe immune-mediated thrombocytopenia secondary to infection with HIV. AIDS. 1991; 5: 1357-61.
17. Moses A, Nelson J, Bagby GC Jr. The influence of human immunodeficiency virus-1 on hematopoiesis. Blood. 1998; 91: 1479-95.
18. Moyle G, Sawyer W, Law M, Amin J, Hill A. Changes in hematologic parameters and efficacy of thymidine analogue-based, highly active antiretroviral therapy: a meta-analysis of six prospective, randomized, comparative studies. Clin Ther. 2004; 26: 92-7.
19. Koka PS, Reddy ST. Cytopenias in HIV infection: mechanisms and alleviation of hematopoietic inhibition. Curr HIV Res. 2004; 2: 275-82.
20. Nair MPN, Schwartz SA. Reversal of human immunodeficiency virus type 1 protein-induced inhibition of natural killer cell activity by alpha interferon and interleukin-2. Clin Diagn Lab Immunol. 2000; 7: 101-5.
21. Nascimento FG, Tanaka PY. Thrombocytopenia in HIV-Infected Patients. Indian J Hematol Blood Transfus. 2012; 28: 109-11.
22. Majluf-Cruz A, Silva-Estrada M, Sanchez-Barboza R, *et al*. Venous thrombosis among patients with AIDS. Clin Appl Thromb Hemost. 2004; 10: 19-25.
23. Barnett JH, Gilson I. Zidovudine-related thrombocytopenia simulating rapid growth of Kaposi's sarcoma. Arch Dermatol. 1991; 127: 1068-9.
24. Jong E, Louw S, Meijers JCM, *et al*. The hemostatic balance in HIV-infected patients with and without antiretroviral therapy: partial restoration with antiretroviral therapy. AIDS Patient Care STDS. 2009; 23: 1001-7.
25. Crum-Cianflone NF, Weekes J, Bavaro M. Review: thromboses among HIV-infected patients during the highly active antiretroviral therapy era. AIDS Patient Care STDS. 2008; 22: 771-8.
26. Fezoui H, Garnier G, Taillan B, Cassuto JP, Pesce A. [Hemostasis anomalies and human immunodeficiency virus infection]. Rev Med Interne. 1996; 17: 738-45.