Clinical and immunological status of a newly diagnosed HIV positive population, in Marrakech, Morocco

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Abstract

Objective: To evaluate the clinical and the immune status of newly HIV diagnosed patients, in Marrakech city and its neighboring area, in Morocco.

Methods: We performed a retrospective study on 235 patients who have been previously confirmed for HIV infection, and underwent a CD4 T cells using flow cytometry (FacsCount, Becton Dickinson®).

Results: The mean age of patients was 34,3 ± 8,4 years (range: 14-55), with a male predominance (sex-ratio M/F=1.4). On basis of clinical data of the patients, 62% (n=146) of them were categorized as "category C", 18.4% (n=43) as "category B", and 19.6% (n=46) as "category A" according to CDC (Center for Disease Control) HIV classification. Among all of them, 60.4% (n=142) had less than 200 CD4T cells, 26% (n=61) had between 200 and 499 CD4T cells, and only 13.6% (n=32) showed a number of CD4T cells less or equal to 500/mm^3.

Conclusion: The results of this study reflect a significant delay in the diagnosis of HIV infected patients. Therefore, this delay may compromise timely management of HIV infected individuals and enhances propagation of the epidemic in our country. These data confirm the need for intensifying prevention efforts among high-risk population. Moreover, continuing education in HIV/AIDS among healthcare providers should be reinforced.

Key words: HIV Infection, CD4T cells count, CDC Classification, Marrakech, Morocco

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Introduction

Human Immunodeficiency Virus (HIV) has a selective tropism for cells which express the CD4+ phenotypic marker: T Lymphocytes, monocytes and macrophages[1]. The main biological event in HIV infection is the immunity system collapse, especially CD4 T cells gradual destruction that lead to a severe immune depression and consequently a high risk of opportunistic infections and cancers[1,2,3]. The CD4 count is a useful tool for the initiation and the follow-up of the anti-retroviral therapy response[1]. The decrease of CD4+ T cells during HIV infection may result from several mechanisms: cytopathogenic effect of the virus on infected cells, CD8+ T lymphocytes mediated cytotoxicity, or abnormality of CD4+T cells distribution or arrest of their renewal[2,4]. During HIV infection, some authors relate two immunological stages depending on CD4+T cells number: the first stage with less than 400 CD4+T cells/mm3, characterized by a deficiency of mucosal immunity is associated to a deficit of skin delayed hypersensitivity. The second stage with CD4+T cells<50/mm^3 includes a systemic immune deficiency, commonly characterized by cytomegalovirus (CMV) and Mycobacterium Avium infections[5].

Other authors attribute the risk of the opportunistic infections to specific degree of immunosupression[6]. Thus, herpes simplex virus (HSV), candida and pyogenic infections may occur in asymptomatic persons, while the risk of pulmonary pneumocystis and about 80% of tuberculosis cases increase significantly when the rate of CD4+T cells is less than 200/mm^3 [6,7]. Similarly, non Hodgkin lymphoma and Kaposi sarcoma or Mycobacterium, CMV and toxoplasmosis infections occur typically when the rate of CD4+T cells is inferior to 100/mm^3 [8,9,10].

In Morocco, since the first case of HIV infection has been diagnosed in 1986, there has been a steady increase of HIV/AIDS cases, and currently there is

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around 20, 000 infected persons.[11]. The diagnosis is mostly done in severe or acute clinical circumstances, or among risk groups, blood donors, or within voluntary counseling and testing (VCT) facilities. Clinical diagnosis was initially provided in few specialized centres belonging to university hospitals. Since 2004, the HIV screening has been expanded by the introduction of rapid testing in VCT centres affiliated to nongovernmental organizations (NGOs) and within HIV referral centres. Moreover, HIV testing using conventional techniques (ELISA and Western blot tests) was decentralized in 20 regional laboratories. This strategy is aimed at enhancing earlier HIV diagnosis and therefore an early HIV management.

In Morocco, Marrakech city and its neighboring area account for 15% of all HIV-infected patients in our country. There are two referral HIV clinical centres, located in the Ibn Zohr regional hospital and in the university hospital center, that are in charge of management of HIV-infected individuals of this area. Furthermore a clinical laboratory, within university hospital centre, which is supervised by the national reference laboratory for HIV, assures the HIV diagnosis as well as CD4 counting.

The aim of this study was to evaluate the clinical and the immune status of newly HIV diagnosed patients of this region according to their clinical stages.

Methods

We performed a retrospective study on 235 newly HIV diagnosed patients, corresponding to all patients admitted over the time between January 2006 and December 2008, within two referral HIV health care centres, located in Ibn Zohr regional hospital and the university hospital centre, in Marrakech. The patients are addressed to the HIV diagnosis and management facilities, since they have symptoms and or risky behaviors. Individuals that screened within the framework of VCT are addressed to these referral HIV care units, as well. The patients mostly came from urban areas and from rural areas. All these patients have been confirmed for HIV infection, within the laboratory of the University Hospital Centre, by using an enzyme immunoassay test (ELISA HIV/1-2, Genscreen plus®) as a screening test, followed by a confirmatory technique i.e. the Western-Blot test (HIV BLOT 2.2 Genlabs Diagnostics®), and patient's samples met the World Health Organization (WHO)[13] Western blot test positivity criteria. According to the clinical symptoms recorded during the first visit, data were recorded in the medical file, and HIV specialist physicians used CDC (Centre for Disease Control) classification system[12] to categorize the clinic stages for all HIV-infected individuals. Finally, to assess the immune status of these patients, a CD4 count is prescribed at the initial visit, and it is measured within a week following the visit. The measurement is preformed by the FacsCount instrumentation, which gives an absolute count of CD4 T cells/ µl (FacsCount, Becton Dickinson®).

Results

Seventy seven percent of patients (n=181) came from urban area, versus 33% (n=54) who was originate from rural districts of Marrakech surroundings. The mean age was 34.3± 8,4 years (range:14-55 years), with a slight male predominance (sex-ratio M/F=1.4). The most age range concerned by the HIV disease was 25-34 years (40%, n=94) followed by 35-44 (35.3%, n=83), 45-55 (16.6%:n=39) and 14-24 (8.1%, n=19) years range respectively. Unmarried individuals were the predominant proportion (44.7%, n=105) in this series (Table 1), and homosexual or bi-sexual behavior was noticed in 19 patients (8.1%) versus 206 patients (87.7%) who were generally heterosexual.

Table 1: Distribution of patients according to socio-demographic characteristics, clinical and CD4+T cell count categories

	Clinical CDC categories			
	Stage A	Stage B	Stage C	Total
	n	n	n	n (%)
Socio-demographic characteristics				
Mean age	33.2	33.5	35,3	34,3
Gender (M/F)*	18/28	29/14	90/56	137/98
Origin (U/R)*	41/5	30/13	110/36	181/54
Marital status (U/M/D/W)*	27/15/3/1	29/8/5/1	49/61/21/15	105/84/29/17

Continuation of table 1

	Clinical CDC categories			
	Stage A	**Stage B**	**Stage C**	**Total**
	n	n	n	n (%)
Diagnosis circumstances				
Common/specific symptoms	12	14	72	98 (41.7%)
Opportunistic infections:	-	16	67	83 (35.3%)
Other circumstances:	32	12	3	47 (20%)
Unknown conditions:	2	1	4	7 (3%)
CD4+ T cell count (cells/mm^3)				
Greater or equal to 500	24 (75%)	3 (9.3%)	5 (15.7%)	32 (13.6%)
200<CD4 Less or equal to 499	18 (29.5%)	27 (44.3%)	16 (26.2%)	61 (26%)
Less or equal to 200	4 (2.8%)	13 (9.2%)	125 (88%)	142 (60.4%)
Total	**46 (19.6%)**	**43 (18.4%)**	**146 (62%)**	**235 (100%)**

* M/F: male/female U/R: urban/ rural. U/M/D/W: unmarried, married, divorced, widowed

The sexual status was not declared by 10 patients (4.2%). Furthermore, 38% of patients (n=89) avowed sexual connection with many partners and the age of the first sex could not be significantly determined. Clinically, in 41.7% of cases (n=98), the infection was revealed by various common or specific symptoms, primarily diarrhea with weight loss or cachexia (Table-2). Opportunistic infections were the form of HIV disclosure in 35.3% of cases (n=83), from which tuberculosis was the most frequent. Moreover, the disease has been uncovered in many other circumstances such as voluntary screening at VCT facilities or others similar conditions (Table 2) among 20% of patients (n=47), while, diagnosis conditions could not be reported in 3% of cases (n=7).

Table 2: Clinical manifestations of HIV at presentation

Clinical data	**(%)**
Common or specific symptoms:	**(41.7%)**
Diarrhea associated to weight loss	(14.4%)
Weakness or cachexia	(5.1%)
Oropharyngeal candidiasis	(4.7%)
Prolonged fever	(3.8%)
Severe pneumopathy or pulmonary abcesses	(3.4%)
Cutaneous infections	(2.5%)
Diffuse and persistent lymphadenopathy	(2.1%)
Varicella	(1.2%)
Oral aphtosis	(0.8%)
Radiculopathy	(0.8%)
Atypical bacterial meningitidis	(0.8%)

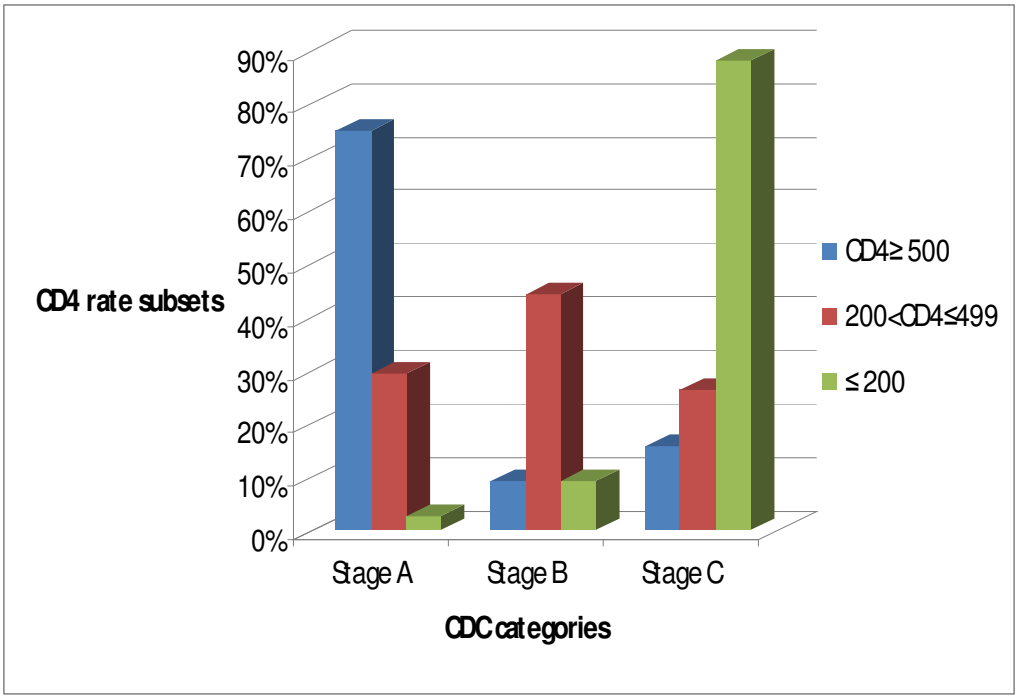
Continuation of table 2

Clinical data	**(%)**
Disseminated dermatophytis	(0.4%)
Gonococcal arthritis	(0.4%)
Pulmonary hypertension	(0.4%)
Neurosyphilis	(0.4%)
Opportunistic infections:	**(35.3%)**
Tuberculosis	(11.4%)
Cryptococcus meningititis	(4.7%)
Oesophageal candidiasis	(3.8%)
Pneumocystosis	(3.4%)
Cerebral toxoplasma	(2.1%)
Kaposi sarcoma	(2.1%)
Recurrent mucosal herpetic infection	(2.1%)
Mycobacterium pneumopathy	(2.5%)
HIV encephalitis	(1.2%)
HSV infection	(0.8%)
Severe zoster infection	(0.4%)
CMV retinopathy	(0.4%)
Other circumstances:	**(20%)**
Voluntary screening	(9.8%)
Testing for sexually transmitted diseases	(5.5%)
Blood donors	(2.9%)
Recruitment examination	(1.2%)
Prenuptial checking	(0.4%)
Unknown conditions:	**(3%)**
Total:	**235**

The clinical data showed that 19.6% (n=46) of patients were diagnosed in stage A, 18.4% (n=43) in stage B, while 62% (n=146) were in stage C of the infection according to CDC classification. We

noticed a predominance of female patients among CDC category A (sex-ratio M/F=0.6), and male gender among in B (sex-ratio M/F=2.1) and C (sex-ratio M/F=1.6) categories. Patients originate from rural area were more frequent in category C than B and A categories. Likewise, the proportion of divorced or widowed patients was significantly more important in category C than B and A categories (p=0.01). The CD4 subset immunophenotyping showed that 60.4% of the patients (n=142) had a CD4 rate inferior to 200/mm3 of which 88% were categorized as stage C, 9% as stage B and 3% were included in stage A. In 26% (n=61) of cases, the number of CD4+T cells was between 200 and 499/mm^3. Forty four percent fell into category B, 29.5% in category A, and 26.2% in category C. Only 13.6% (n=32) of the patients had a number of CD4 superior or equal to 500/mm^3 of which 75% were at stage A, 15.7% at stage C and 9.3% at the stage B (Table 1 & Figure 1). The CD4+T cells average for all the patients was 225/mm^3.

Figure 1: Distribution of CD4$^+$T lymphocyte rate subsets among HIV-infected CDC categories



Discussion

The results of this series show that 48% of HIV infected population had less than 35 years, and the majority had symptoms of AIDS disease that indicate the onset or existing opportunistic infections. Also, more than half of patients were diagnosed in an advanced stage of immunosupression linked to HIV disease, since 60.4% of them had less than 200 CD4+Tcells/mm^3, 26% had a CD4+T cell number ranging between 200 and 499/mm^3 and only 13.6% kept a rate of CD4+ T superior or equal to 500/mm^3, with an average of 225 CD4/mm^3. As shown in the results, 88% of cases with less than 200 CD4+T cells/mm3 were matching with category C of CDC classification system. In fact, a lower CD4 rate in HIV infected individuals may give evidence that the patient has been infected for a long time as compared to those with higher CD4 count[14]. However, primary HIV infection can be accompanied by a profound transient lymphocytopaenia including low CD4+T cells, and opportunistic infections may occur at this stage, but these infections should not be confused with clinical staging events developing in established HIV infection[15,16]. This fact might explain the low rate of CD4+T cells in 4 (2.8%) of patients belonging to category A in our series. Moreover, in acutely HIV-infected patients 4–6 weeks post-infection, there is usually a depletion of the gut associated lymphoid tissue (GALT) limited to a predominantly CD4+T cell subsets exhibiting a memory phenotype [CD45RA$^-$/CD45RO$^+$]. This depletion is attributed to preferential replication of HIV-1 in the GALT where CD4 are the predominant residing T cells[17]. Likewise, Diagbouga showed that the early depletion of CD4+T cell is

mainly due to a decrease of CD4+CD29 ^{high} subset, whereas CD4+CD45RA+ phenotype is unaltered in CDC-A stage of the disease. But, in the later stages (B and C), both CD4+CD29^{high} and CD4+CD45RA+ subsets contribute to CD4 T cell depletion[18]. In all of cases, individuals with less than 200 CD4/mm^3 may have both poorer response to anti-retroviral therapy and worse prognosis than those with higher CD4 count at the time of therapy initiation[19].

In Morocco, even with the current availability of HIV testing program, expansion of testing in medical settings and VCT centers, the delay in diagnosis was noticed in our population. This might be related to a weak adhesion to these efforts, and to difficulties to access health care system for some populations, especially who come from rural area. A late diagnosis may also be due to a relatively weak knowledge among medical staff towards HIV associated diseases.

Moreover, the adverse image of HIV infected person in our society usually leads to some reticence towards both the diagnosis and the monitoring on regular basis**.** It is worthwhile noting that regarding to HIV epidemic, the HIV/AIDS program department in the Moroccan health ministry has set up a national strategy aimed to curb the epidemic in the kingdom. This strategy primarily encompasses prevention and strengthening of the early HIV diagnosis as well as treatment of HIV-infected patients[20]. Generalizing free access to HIV testing throughout the country represents the main priority, since 2008. In this regard, and with the efforts of the Moroccan Ministry of Health, the HIV testing is being available in many basic health care points, in Morocco. The results of our study are relatively comparable to some African studies, which also display a delay in the diagnosis of HIV infected patients. Thus, in a Benin study including 136 initially diagnosed and monitored patients between 2001 and 2002, 46.3% of them were in stage C, and 69% of cases had less than 200 CD4 T/mm^3 [21]. Furthermore, a French study carried out in Saint-Antoine hospital in Paris between 2002 and 2003 concerning 300 new cases of HIV infection, from different origins: 43% Caucasian, 44% African, 8% North African and 3% Asian. The average of CD4+T cell was 374/mm^3. Only 23.3% of the French had less than 200 CD4/mm^3 versus 40% among patients coming from Sub-Saharan Africa. It was concluded that the delay of HIV diagnosis is important among African emigrants in France, and may be favored by cultural and socioeconomic context[22]. Likewise, a multicenter study carried out in the United Kingdom in 2003, has found similar results. In fact, a significant number of emigrants originated from sub-Saharan Africa who are diagnosed at an advanced HIV infection stage, with less than 200 CD4/mm^3 [22,23]. On the other hand, our results reflect an obvious delay in the management of patients in comparison with European and North American studies.

A multicenter study conducted in 17 hospitals of the North and North East of France regarding about 595 newly HIV diagnosed patients, displayed that the majority of those patients (75%) were totally asymptomatic (category A), 10% belonged to the category B and about 15% had AIDS-defining conditions (category C) [24]. In addition, it was suggested that the variation of virus subtypes may also contribute to some discrepancies in HIV progression between continents[25]. Some authors suppose that subtype E commonly seen in some Asian countries like Thailand and India seems to be a lot more virulent than subtype B which is predominant in Europe and United States of America[25,26].

In a series of 235 newly HIV diagnosed Thai patients with 96% of recombinant A/E form and 4% of subtype B, Ruxrungtham and al[24] showed an average of CD4+T cells inferior to 200/mm^3, with a 5-year survival of 82% for group A/E, versus 90% for group B[26]. A Moroccan study carried out between 1993 and 1996 has shown a predominance of sub-type B (93.5%) which is in accordance with the occidental pattern of HIV diversity. Nevertheless, another study conducted between 2001 and 2005 displayed a large genotypic diversity of HIV subtypes in Morocco with emergence of non B subtypes (34%) and recombinants circulating forms [27,28]. In our present work, the assessment of HIV diversity consequence on the progression of HIV infection and its immunologic impact has not been studied. Such impact may be important to be explored.

Conclusion

The results of our study showed a delay in the diagnosis of HIV infected patients and confirm the need for intensifying prevention actions, expansion and promotion of HIV testing in healthcare facilities and within voluntary counseling and testing centres affiliated to NGOs. Further studies assessing the role of other factors (cultural, psychological, socioeconomic) may help understanding the cause

of late HIV diagnosis, and should be undertaken in order to improve the management of HIV infection in our country.

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References

1. Gupta SB, Gilbert R.L, Brady AR, Livingstone SJ, Evans BG. CD4 cell counts in adults with newly diagnosed HIV infection: results of surveillance in England and Wales, 1990-1998. *AIDS* 2000 May; 14(7): 853-61.
2. Lelièvre JD, Arnoult D, Petit F, Estaquier J. Infection par le VIH-1 et apoptose lymphocytaire T CD4. *La revue de Médecine interne* 2003; 24 : 522-9.
3. Garrait V., Molina J.M. Nouvelles stratégies de traitement anti-rétroviral chez les patients infectés par le VIH. *Pathol Biol* 2001; 49: 67-71.
4. Napolitano. Approach to immune reconstitution in HIV infection. Perspective. International AIDS Society-USA 2003 September/October; 11(5):160-6.
5. Mallal SA. The Western Australian HIV Cohort Study, Perth, Australia. *J Acquir Immune Defic Syndr Hum Retrovirol.* 1998; 17 Suppl 1: 23-7.
6. Moore RD and Chaisson RE. Natural History of Opportunistic Disease in an HIV-Infected Urban Clinical Cohort. *Annals of Internal Medicine* 1996 April; 124(7): 633-42.
7. Lesprit Ph et Molina J-M. La co-infection VIH-tuberculose : causes et conséquences *Méd Mal Infect* 1996; 26: 918-21.
8. Morlat PH, Ragnaud JM, Gin H, Lacoste D, Beylot J and Aubertin J. La toxoplasmose cérébrale au cours du SIDA. *Médecine et Maladies Infectieuses* 1993 Février;23 Suppl1:183-9
9. May T, Rabaud C, Katlama C, Leport C, Ambroise-Thomas P, Canton Ph. and le groupe d'étude. Toxoplasmose extra-cérébrale au cours du SIDA : résultats d'une enquête nationale Médecine et *Maladies Infectieuses* 1993 February ; 23, Suppl 1 :190-200.
10. Lafeuillade A, Poizot-Martin I, Boulat O, Allegre T, de Jaureguiberry JP, le Groupe d'Etudes en Médecine Interne de Provence (GEMIP). Le sarcome de Kaposi de présentation extracutanée au cours du SIDA : 24 observations. *Médecine et Maladies Infectieuses* 1994 February; 24(2): 92-95.
11. Karkouri M. L'infection à VIH au Maroc. *Annales de pathologie* (2008) 28S, 113
12. Castro K-G, Ward J-W, Slutsker L, Buehler J-W, Jaffe H-W, Berkelman R-L. 1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS Among Adolescents and Adults. Recommendations and reports. http://www.cdc.gov.
13. WHO. Proposed WHO criteria for interpreting results from Western blot assays for HIV-1 and HIV-2 and for HTLV-I /HTLV-II Weekly epidemiological record 1990; 65(35): 281-83.
14. Stein D.S., Korvick J.A., Vermund S.H. CD4+ lymphocyte cell enumeration for prediction of clinical course of human immunodeficiency virus disease: a review. *J Infect Dis.*1992; 165(2):352–63.
15. Soogoor M, Daar ES. Primary HIV-1 Infection: Diagnosis, Pathogenesis, and Treatment. *Curr Infect Dis Rep.* 2005 Mar; 7(2):147-53.
16. Douek D, HIV Disease Progression: Immune Activation, Microbes, and a Leaky Gut. International AIDS Society–USA *Topics in HIV Medicine* 2007; 15 (4):114-17.
17. Centlivre M, Sala M, Wain-Hobson S, Berkhout B. In HIV-1 pathogenesis the die is cast during primary infection. *AIDS* 2007; 21:1–11.
18. Diagbouga S, Chiron JP, Sanou O, Ledru E. Alteration in CD29high CD41 Lymphocyte Subset is a Common Feature of Early HIV Disease and of Active Tuberculosis. Scand. *J. Immunol.* 2001; 53: 79-84.
19. Sterling TR, Chaisson RE, Keruly J, Moore RD. Improved outcomes with earlier initiation of highly active antiretroviral therapy among human immunodeficiency virus infected patients who achieve durable virologic suppression: longer follow-up of an observational cohort study. *J Infect Dis.* 2003; 188(11):1659–65.
20. Plan stratégique national de lutte contre le Sida 2007-2011, DELM. Ministère de la Santé, Royaume du Maroc. www.sante.gov.ma
21. Zannou D.M., Kindé-Gazard D., Vigana J., Adè G., Sèhonou J.J., Atadokpèdé F., Azondékon A., Gbèdo C., Anagonou S.Y. and Houngbé F. Profil clinique et immunologique des patients infectés par le VIH dépistés à Cotonou, Bénin. *Médecine et Maladies Infectieuses* 2004 May ; 34(5) : 225-228.
22. Fonquernie L, Dray-Spira R., Bamogo E, Lert F. and Girard P.M. Caractéristiques des patients nouvellement pris en charge pour une infection

VIH dans un CHU parisien en 2002–2003. *Médecine et Maladies Infectieuses* 2006 May; 36(5) : 270-279.

23. Boyd AE, Murad S, O'shea S, de Ruiter A, Watson C., Easterbrook P.J. Ethnic differences in stage of presentation of adults newly diagnosed with HIV-1 infection in south London. *HIV Med.* 2005 Mar; 6(2):59-65.
24. Doco-Lecompte T, Yazdanpanah Y., Fischer P., Froidure M., Douadi Y., Strady C., Chirouze C., Rabaud C. Infections à VIH nouvellement découvertes dans le nord et le nord-est de la France : données 2000 et 2001. *Médecine et maladies infectieuses* 2003;33:304–8.
25. Wannamethee SG, Sirivichayakul S, Phillips AN, Ubolyam S, Ruxrungtham K, Hanvanich M, Phanuphak P. Clinical and immunological features of human immunodeficiency virus infection in patients from Bangkok, Thailand. *International Journal of Epidemiology* 1998; 27:289-95.
26. Ruxrungtham K., Phanuphak P. Update on HIV/AIDS in Thailand. *J Med Assoc Thai* 2001 Jun; 84 Suppl 1:11-17.
27. Elharti E, Alami M, Khattabi H, Bennani A, Zidouh A, Benjouad A, El Aouad R Some characteristics of the HIV epidemic in Morocco. *East Mediterr Health J.* 2002 Nov;8(6):819-25.
28. Papathanasopoulos M-A, Hunt G-M. and Tiemessen C-T. Evolution and Diversity of HIV-1 in Africa – a Review. Virus Genes 2003 March; 26(2): 151-63