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HIV Viral Load Testing in Laos

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Abstract. The number of people living with HIV/AIDS in the People's Democratic Republic of Laos (also known as Laos PDR) is estimated at 13,600 and the number of people in need of antiretroviral therapy at 8,000. Today, around 3,200 HIV infected individuals receive treatment in seven centres throughout the country. Until recently, antiretroviral treated patients were followed-up only on the basis of clinical and immunological criteria.

In 2009 the Centre d'Infectiologie Christophe Mérieux in Laos PDR (CICML) signed a collaboration agreement with the national Centre of HIV/AIDS/STI (CHAS) for the implementation of HIV viral load testing (VLT) in the country, leading to the technological transfer of the ANRS generic assay (HIV Generic charge virale, Biocentric, Bandol, France). The introduction of HIV VLT has been accompanied through national HIV workshops every 6 months. From June 2009 to December 2011, HIV viral load has been measured in 1,782 antiretoviral-treated patients. Of these, 97% were on reverse-transcriptase inhibitor -based 1st line regimen.

HIV viral load was undetectable (<250 copies/ml) for 1,491 out of 1,782 (84%) antiretroviral-treated patients. Four months after adherence strengthening, HIV viral load became undetectable for 179/247 patients (72.5%) while 68/247 patients (27.5%) remained viremic (median viral load: 4.1 Log_{10}).

This report demonstrates the feasibility of setting up an affordable HIV viral load generic test at a national level in Laos PDR. Interestingly, only 16% of antiretroviral-treated patients presented with detectable VL at their first viral load measurement. Importantly, almost two thirds of them have controlled their viral load after strengthening their adherence to treatment.

Keywords. HIV/AIDS, Laos, viral load, antiretroviral therapy

1. Introduction

The Laos People's Democratic Republic (Laos PDR) is a landlocked country in Southeast Asia, bordered by the Republic of the Union of Myanmar and the People's Republic of China to the northwest, Vietnam to the east, Cambodia to the south and Thailand to the west.

Spread over 236,800 km², with 6.8 million inhabitants in 2009, the country has a population density of 28.8inhabitants perkm²; 69% living in rural areas and 31% in urban areas. Laos PDR is divided into 17 provinces further divided into districts and then villages. The capital city is Vientiane (350 000 inhabitants). Other large cities include Savannakhet (130,000 inhabitants), Pakse (70,000 inhabitants) and LuangPrabang (60,000 inhabitants).

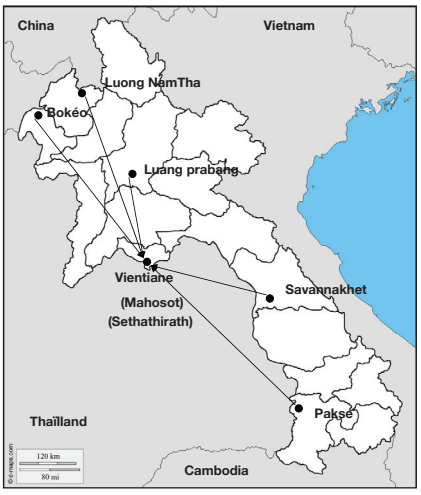

Figure 1. Map of Laos PDR and location of the seven ART treatment centres

1.1 Dynamics of HIV infection in Laos PDR

The Laos PDR is often considered as the country in the Greater Mekong Region with the lower HIV-prevalence in the general population. However, the Laos PDR is undergoing rapid socioeconomic changes, leading to behaviours that may place some Laotians at increased risk of HIV infection. Because of its geographical location in the heart of the Mekong region, intravenous drug use, and unsafe sexual practices, Laos PDR is at high risk of a greater epidemic. Since the first HIV case was identified in 1990, the number of infections has constantly increased. Although only less than 1% of sex workers were HIV-infected in 2000, a 2004 survey of the prevalence of sexually transmitted infections (STIs) among service women found that chlamydia /gonorrhoea prevalence was 45% in the capital Vientiane, 43.6% in the border province of Bokeo, and 27.9% in the southern province of Champasak, indicating the vulnerability of these women to HIV (UNAIDS, 2010). In 2005, nearly 5% of injecting drug users (IDUs) were found HIV infected and UNAIDS estimated that 3,700 people in Laos PDR were living with HIV. In 2009, the estimated HIV prevalence among 15 to 49 year old was close to 0.2% giving an estimated number of people living with HIV/AIDS (PLWHA) of 13,600, according to UNAIDS (2010).

1.2 An increasing number of patients on antiretroviral therapy (ART)

In response to its precarious HIV situation, the Government of Laos PDR has provided strong political commitment to support a multi-sectorial response. The action of key international and national partners has been also invaluable. Médecins Sans Frontières (MSF) opened its HIV/AIDS project in Savannakhet, southern Laos PDR, in 2001. Along with providing urgently needed treatment, the project aimed to sensitize the general public and the authorities to the existence of HIV/AIDS and the need for specialized care. MSF's project offered free services for patients, who until recently travelled from all provinces to benefit from them. In Savannakhet, MSF provided prophylaxis and treatment for opportunistic infections and ART. By 2007, HIV counselling, testing and ART services had been handed over to the hospital. By the end of 2007, 608 patients were receiving care and treatment, with 490 of them on ART.

In spring 2005, the Ministry of Health of Laos PDR requested the support of the French public interest group "Ensemble pour une solidarité thérapeutique hospitalière en réseau" (ESTHER) ("Together for a therapeutic solidarity network hospital"). An agreement between the two countries was signed in April 2007. The partnership project between Mahosot Hospital in Vientiane, Saint-Louis Hospital, Assistance Publique/ Hopitaux de Paris (APHP) in Paris, and Strasbourg University Hospital, along with the hospital members of the Program for HIV Prevention and Treatment (PHPT) network in Chiang Mai, began in early 2008. This project, planned for a 5 year period (2008 - 2012), has been co-funded by the French Development Agency (AFD) as part of its health project in Laos PDR. The partnership project between the Luang Prabang Provincial Hospital, which became the 4th ART centre in the country in February 2009, and Bichat-Claude Bernard Hospital-APHP got underway in March 2009. The main objective was to strengthen the capacities of the health care system at national level and in both hospitals, through mentoring, training workshops and exchange of practical experience. Access to viral load was improved through the financing of viral load tests in Mahosot and Luang Prabang Hospital.

To date, seven centres have been gradually established: two in Vientiane (Mahosot, Setthatirath), 3 in the north (Luong NamTha, Bokeo, Luang Prabang) and two in the south (Savannakhet and Pakse) (Figure 1). At the end of 2011 the number of patients receiving ART was estimated at 2,115.

1.3 Lack of virological monitoring

Despite the aforementioned accomplishments, Laos PDR had still many challenges to address including the lack of virological monitoring to follow antiretroviral-treated (ART) patients. Indeed, before 2009, monitoring of patients on ART was done only on the basis of clinical and immunological (CD4 lymphocytes count) criteria and only very few patients had access to HIV viral load measurement in Thailand for USD 200 to 250 with results released after 4 to 8 weeks. In the absence of viral load measurement, the early diagnosis of HIV infection in newborns and children under18 months was impossible.

2. HIV viral load implementation in Laos PDR

The Centre d'Infectiologie Christophe Mérieux of Laos PDR (CICML) was established in 2009 with a mission to serve public health. Today, the CICML is integrated into the Laos public health infrastructures and is under the guidance of the Laos Ministry of Health.

Within the year, the CICML signed an agreement with the national Centre for HIV/AIDS/STI (CHAS) for the introduction of HIV viral load testing (VLT) in the country. The CICML has capitalized on the expertise of IPC for the implementation of the HIV viral load using the generic test developed by the French Agence Nationale de Recherche sur le Sida et les hépatites virales (ANRS) (Rouet et al, 2007; Rouet et al, 2008) and commercialized by Biocentric, France. This generic test, based on real time technology, targets the long terminal repeat (LTR) region of HIV which allows quantification of the vast majority of HIV-1M strains circulating around the world including HIV-1 non-B viruses such as the CRF01_AE subtype circulating in South-East Asia (Menu et al, 1999; Nouhin et al, 2009).

This generic assay is also particularly sensitive with a limit of detection of 50 copies/ml using 1 ml plasma, or 250 copies/ml using 200 microlitres of plasma (Rouet et al, 2008). In contrast to the other commercialized tests, the Biocentric assay uses an open system that offers great flexibility in the choice of reagents, consumables and equipment required for both the nucleic acid extraction and the amplification/detection steps. Based on real-time PCR, the risks of nucleic acid contamination are greatly reduced. Routinely used in clinical

trials conducted by the ANRS, in France and also in southern countries, such as in Cambodia and Africa (Kesho Bora Study Group, 2011; Blanc et al, 2011), it has been gradually implemented in around 20 African and Asian countries. This approach significantly reduces the cost of this analysis to USD 20 to 40 including reagents, transport, staff, and consumables.

In Cambodia, both HIV-RNA and HIV-DNA VL assays (Generic HIV charge virale and HIV DNA cell, Biocentric, Bandol, France) were implemented at Pasteur Institute Cambodia (IPC) in 2005. Between 2005 and 2011, 28,000 HIV RNA VL have been done for 13,000 HIV patients representing one third of the ART- patients in Cambodia. During the same period of time, nearly 3,700 exposed infants less than 18 months of age have been screened for HIV infection by detection of HIV-RNA on whole blood or HIV-DNA on dried blood spot (DBS).

2.1 The Technical transfer:

It was clear to all partners that the training of the laboratory staff working at the CICML should be also accompanied by 1) providing training to clinicians on the use and interpretation of HIV viral load, 2) establishing national logistics for the specimen collection/transportation and data feedback to the sites, and 3) warranting the sustainability through ensuring the funding of viral load at the national level.

The technology transfer of the viral load from IPC to CICML occurred between May and September 2009 during missions of Laos technicians to IPC and Khmer technicians from IPC to CICML. During these missions, intra and inter laboratory quality controls were established, followed-up by registration of CICML to international external quality controls such as Quality Control for Molecular Diagnostics (QCMD), United Kingdom National External Quality Assessment Service (UKNEQAS), and now US Centre for Disease Control and Prevention (US CDC).

To accompany the introduction of this analysis in Laos PDR, it was decided to organize semi-annual national workshops involving representatives of CHAS, WHO, ESTHER, associations of people living with HIV/AIDS, laboratory staff and clinicians from the seven treatment centres, and invited experts in the fields of virology, antiretroviral therapy, and psycho-social sciences. It was decided that each workshop would include a session dedicated to clinical case studies. These workshops, co-organized by the CICML and IPC were financially supported by the national program through the Global Fund, ESTHER and Fondation Mérieux.

2.2 The national workshops on HIV viral load

2.2.1 The first national workshop

Between May and October 2009, a total of 144 viral loads were carried out. The first national workshop on HIV Viral load in Laos PDR was held in Vientiane in November 2009. This meeting provided the opportunity to make the authorities and other stakeholders aware of the availability of HIV VLT in Laos PDR.

It also helped to set up logistics for the transportation of treatment site samples to the CICML. It was decided that frozen plasma specimen from the North (Bokeo, Luang Prabang and Luong Namtha) would be sent once a week by plane to Vientiane, and that plasma specimen from the south (Savannakhet and Pakse) would be sent to the CICML after 8 to 11 hours by bus each week. EDTA blood specimen from Mahosot and Setthatirath in Vientiane would be referred at room temperature and on a weekly basis directly to the CICML.

All samples are sent anonymously with a unique patient code. After receipt and registration, plasma samples are stored at -80 °C until use. All specimens are supposed to arrive at the beginning of the week. Through this method, CICML can run the test (extraction, amplification/detection) at the end of the same week in order to release the results at the beginning of the following week.

These results are sent by fax or electronically by email to the site managers. The original results are sent back to the sites by carriers or identified couriers. Thus, the time between receipt of samples at CICML and receipt of the results by the sites should not exceed 7 to 10 days.

During the first workshop, the funding of viral loads by the national program through the Global Fund was discussed in order to guarantee the sustainability of the action. Similarly, discussions started for the elaboration of national guidelines on virological monitoring of HIV infected subjects.

2.2.2 The second national workshop

The second national workshop was held 6 months later in Vientiane in May 2010. It has been an opportunity to see, that despite the established procedures, the number of samples received at CICML was lower than expected: between the first and the second workshop, only 214 viral loads had been performed.

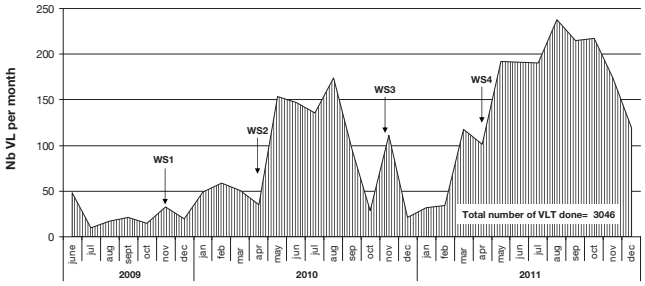

Figure 2. Number of HIV VL tests per months performed at the CICML. From June 2009 to December 2011, a total number of 3,046 tests have been done. WS1-4 means Workshop 1- 4.

Discussions with partners have revealed that one of the 7 centres, managing a large number of patients referred very few samples at the CICML. The measures put in place helped to correct this, and to triple the number of viral loads performed between the second and the third workshop.

2.2.3 The third national workshop

This workshop, held in Vientiane in November 2010, focused on HIV-drug resistance. This workshop was an opportunity to present the ARV resistance testing transferred from IPC to CICML and to emphasize the need to strengthen the patients' adherence to ART and to monitor their viral load 3 months after a first detectable VL, at least, before considering any treatment change or switching to protease inhibitor (PI)-based second line. A special session was dedicated to the psychosocial support and adherence to treatment with the participation of two experts in social sciences and representatives of PLWHA in Laos PDR.

2.2.4 The fourth national workshop

This workshop was held in Luang Prabang in May 2011. As shown in Figure 2, a highly significant decrease in the number of VL done at the CICML was observed between November 2010 and May 2011 (417 HIV VL compared to 735 performed during the previous period). This point was discussed during the 4th Workshop. In fact, the lack of funding at the national level had indeed temporarily slowed down the viral monitoring activity. With this problem solved, the CICML reached its cruising workload with an average of 200 tests per month. The 4th workshop was also an opportunity to introduce the technical transfer of the early infant diagnosis of HIV infection on DBS from IPC to CICML and to emphasize the need to strengthen urgently the Prevention of Mother-to-child HIV Transmission (PMTCT) programs in the country.

3. Virological efficacy of antiretroviral therapy

From May 2009 to December 2011 the CICML received a total of 3,056 blood specimen corresponding to 1,782 patients on ART out of the 3,209 recorded in the seven treatment centers.

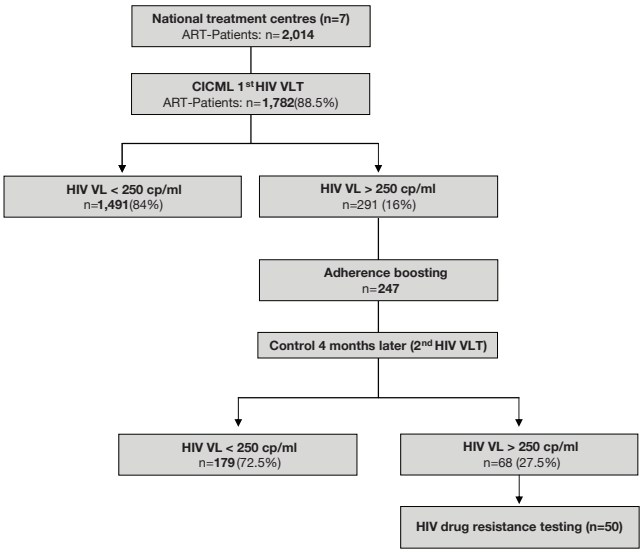

Figure 3. Patients and results flow chart

Savannakhet and Setthatirath are the larger treatment centres with respectively 1,065 and 1,150 patients registered. As indicated in Figure 3, at least one VL was done for 1,782 out of 2,014 patients on ART (88.5%) (Paboriboune et al, 2012). The proportion of patients on ART who received at least one viral load measurement ranged from 66.3% in Luang Prabang to 89% in Bokeo. The median age was 35 years (range: 0.4 – 71), 848 (47.6%) were females and 934 (52.4%) were males.

Among the 1,782 patients on ART with at least one VLT, 1,734 (97%) received reverse transcriptase inhibitor (RTI)-based 1st line regimen (mean duration on1st line: 2.7 years, standard deviation: 1.9) with 988 (57%) receiving d4T/3TC/NVP, 312 (18%) AZT/3TC/NVP and 191 (11%) d4T/3TC/EFV (other RTI-based regimen: 14%).

Nearly 3% out of 1,782 were on PI-based 2nd line regimen (d4T/3TC/LPV/r: 59%; 3TC/TDF/LPV/r: 21%; AZT/3TC/LPV/r: 10%, other: 10%). The mean duration on PI-based 2nd line regimen was 2.9 years (standard deviation: 2.1).

As indicated in Figure 3, the first HIV VL testing revealed that only 291 (16%) of 1,782 patients on ART had detectable viral loads (mean HIV VL: 3.5Log_{10}), 7 of them receiving a PI-based 2nd line regimen. This means that 84% of ART-patients had successful virological suppression.

The adherence to treatment was strengthened by psychosocial counselors for 247 of the 291 viremic patients followed by a control viral load four months later.The second HIV VL became undetectable for 179 (72.5%) out of 247, while 68/247(27.5%) kept unsuppressed VL (mean HIV VL: 4.1Log_{10}) (Paboriboune et al, 2012). Drug resistance testing done for 50/68 showed a high level of resistance to 3TC and NVP/EFV, reverse transcriptase inhibitors used in the 1st line therapy (83-92%).

4. Discussion

We report here the successful implementation of HIV viral load testing at the CICML, in Vientiane, the capital city of Laos PDR, offering access to virological monitoring of nearly 90% of patients on ART, followed-up in the 7 ART centers of the country.

The participation of all stakeholders in the fight against HIV/AIDS is a key factor in this success. The feasibility and interest of open systems to manage HIV viral load at country level is confirmed. The flexibility, offered by such open systems in the choice of complementary equipments or reagents for nucleic acid extraction and amplification, allows reducing significantly the cost of this analysis compared to the tests commercialized today. Such open systems could be also useful in the future for the quantification of other viruses such as hepatitis B and C, particularly endemic in the country.

The success of this initiative is also due to the establishment of periodic national HIV workshops. The dynamic induced by these meetings allows all partners and stakeholders in the fight against HIV/AIDS to be kept informed of the use of this viral marker across the country, to be aware of problems and to conduct rapid corrective actions. There is no doubt that the active participation of clinicians, laboratories,

members of associations representative of people leaving with HIV and the involvement of national and international experts, contribute to improving the medical care and treatment of patients in Laos PDR. Preliminary results show 84% of virological success. This good result is close to that estimated in other limited resources countries, for example, in neighbouring Cambodia (Janssens et al, 2007; Ségéral et al, 2011; Ferradini et al, 2007). This should encourage health authorities to intensify their efforts in the use of viral load. These results highlight the importance of continually enhancing the adherence of patients to their treatments, to reduce the emergence of resistant viruses.

HIV RNA/DNA viral load and antiretroviral resistance testing are now operational at the CICML. Health authorities may rely on that expertise to strengthen the fight against HIV/AIDS to contain HIV spreading across the country. Indeed, only 3,000 on the estimated 8,000 individuals living with HIV in need of ART are currently registered and followed up in treatment centres. Another challenge will be to improve the prevention of mother to child transmission of HIV and to prevent the spread of HIV in populations at risk.

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