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Sympathetic nervous system function in HIV-associated adipose redistribution syndrome

Petra J. van Gurp[a], Cees J. Tack[a], Marc van der Valk[c], Peter Reiss[d], Jacques W.M. Lenders[a], Fred (C.)G.J. Sweep[b] and Hans P. Sauerwein[e]

It was recently suggested that HIV-associated adipose redistribution syndrome (HARS) results from an autonomic dysbalance. We investigated the local and global sympathetic nervous system function of patients with HIV-1 infection and HARS. Interstitial noradrenaline concentrations in skeletal muscle and subcutaneous adipose tissue were increased in the absence of changes in global sympathetic nerve activity, consistent with locally increased sympathetic activity. This could promote localized lipolysis in subcutaneous adipose tissue and contribute to the development of HARS.

According to a recent hypothesis HIV-associated adipose redistribution syndrome (HARS) may result from an imbalance between sympathetic and parasympathetic tone within subcutaneous and visceral adipose tissues, mediated by antiretroviral therapy-induced selective damage of autonomic pathways [1]. In particular, a relative dominance of sympathetic over parasympathetic tone in subcutaneous adipose tissue could induce selective subcutaneous fat loss.

To test the hypothesis that HARS results from differential changes in sympathetic nervous system (SNS) activity to relevant tissues such as fat and muscle, we studied three groups of male subjects: seven HIV-1-infected individuals with HARS who had previously participated in a trial assessing the effect of protease inhibitor withdrawal on HARS [2–4] (HARS patients, currently treated with three nucleoside analogue reverse transcriptase inhibitors, all with HIV-1 RNA < 50 copies/ml), seven age and body mass index-matched healthy volunteers (control subjects), and seven similarly matched asymptomatic, therapy-naive, HIV-1-infected patients (HIV patients).

We measured interstitial noradrenaline levels in the periumbilical subcutaneous adipose tissue and skeletal muscle tissue (quadriceps of the right leg), using microdialysis to provide an index of selective fat and muscle sympathetic activity [5]. Global SNS activity was assessed by muscle sympathetic nerve activity (MSNA, microneurography) and the measurement of arterial and venous plasma noradrenaline [6]. Cardiovascular sympathetic activity was measured by power spectral analysis of the heart rate and systolic blood pressure. All measurements were performed under baseline conditions and during sympathetic stimulation (lower body negative pressure of -25 mmHg for 30 min or cold pressor test).

In HARS patients, global sympathetic activity as reflected by plasma noradrenaline levels and power spectral analysis was normal. However, sympathetic nerve traffic (MSNA) was lower (Table 1).

At the tissue level, HARS patients had a significantly higher skeletal muscle noradrenaline concentration than control subjects (1.51 ± 0.38 versus 0.74 ± 0.10, $P < 0.05$, Table 1). The subcutaneous adipose tissue noradrenaline concentration also tended to be higher in HARS patients (1.96 ± 0.72 versus 0.83 ± 0.23 nmol/l, $P = 0.07$, Table 1). The muscle/fat noradrenaline ratio (M/F NA) was significant lower in HARS patients compared with control subjects (M/F $NA_{\text{HARS patients}}$ 0.88 ± 0.19 versus M/F $NA_{\text{control subjects}}$ 1.75 ± 0.33, $P < 0.05$), indicating relatively high noradrenaline levels in subcutaneous fat tissue, compared with skeletal muscle.

For all indices of both global and local sympathetic activity, except for venous noradrenaline levels, HIV patients showed similar results to the control subjects.

In response to sympathetic stimulation, plasma noradrenaline concentrations increased significantly in all three groups. This increase was similar in HARS patients and control subjects. In response to sympathetic stimulation by the cold pressor test, MSNA increased in both groups, but the increase was larger in the HARS patients (Table 1).

Muscle and adipose tissue noradrenaline levels did not change significantly in response to lower body negative pressure, neither did the muscle/fat noradrenaline ratio in HARS patients.

The results of this study indicate that the SNS activity in muscle and subcutaneous adipose tissue is increased in HARS patients, but not in HIV-infected patients without HARS. The overall whole-body and cardiovascular SNS activity is normal in HARS patients.

Up to now, only a few studies have reported on SNS activity in HIV patients or HIV patients with HARS, and have reported conflicting results. Mittal *et al.* [7] recently showed a reduced heart rate variability in asymptomatic, therapy-naive HIV-1-infected individuals. In contrast,

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Table 1. Measurement of sympathetic nerve activity.

	HARS patients		HIV patients		Control subjects	
	Baseline	Stimulation	Baseline	Stimulation	Baseline	Stimulation
Global activity						
Power spectral analysis						
SBP MF/total var	0.36 ± 0.08	0.26 ± 0.05	0.25 ± 0.05	0.31 ± 0.05	0.31 ± 0.05	0.21 ± 0.05
RRi MF/total var	0.27 ± 0.06	0.39 ± 0.13	0.34 ± 0.06	0.34 ± 0.08	0.27 ± 0.04	0.20 ± 0.05
Noradrenaline (nmol/l)						
Arterial	1.52 ± 0.14	2.63 ± 0.33[a,d]	1.34 ± 0.16	2.21 ± 0.14[d]	1.28 ± 0.08	1.83 ± 0.11[d]
Venous	1.68 ± 0.21[c]	2.67 ± 0.36[d]	1.23 ± 0.07[b]	2.26 ± 0.23[d]	1.57 ± 0.09	2.24 ± 0.10[d]
Local activity						
Noradrenaline (nmol/l)						
Skeletal muscle	1.51 ± 0.38[a,c]	1.84 ± 0.24	0.81 ± 0.04	1.45 ± 0.33	0.74 ± 0.10	1.22 ± 0.17[d]
Adipose tissue	1.96 ± 0.72	1.98 ± 0.71	0.73 ± 0.11	1.21 ± 0.51	0.83 ± 0.23	0.93 ± 0.31
MSNA (bursts/100 beats)	37 ± 1[a,c]	50 ± 2	43 ± 3	50 ± 2	48 ± 2	53 ± 2

HARS, HIV-associated adipose redistribution syndrome; MF/total var, mid-frequency/total variance; MSNA: muscle sympathetic nerve activity; Rri, RR interval; SBP, systolic blood pressure. Data expressed as means ± SEM.
[a]HARS patients versus control subjects: $P < 0.05$.
[b]HIV patients versus control subjects: $P < 0.05$.
[c]HARS patients versus HIV patients: $P < 0.05$.
[d]Baseline versus stimulation: $P < 0.05$.

Becker *et al.* [8] found no difference in any heart rate variability parameter in HIV-infected patients. Our results obtained by combining different techniques, do not provide any evidence of an increased SNS activity at the whole-body level in this group of patients.

The MSNA of HARS patients was significantly lower compared with both groups. The decreased MSNA at the peroneal nerve may reflect a more generalized decrease in sympathetic nerve traffic activity. Another explanation is that the lower MSNA level is caused by sympatho-inhibition by the increased interstitial noradrenaline levels.

The interstitial concentration of noradrenaline in subcutaneous fat relative to that in skeletal muscle tended to be higher in HARS patients, consistent with an increased noradrenaline content in subcutaneous fat, compared with the other groups. This finding may be consistent with a relatively local sympathetic overactivity in HARS patients, particularly within subcutaneous adipose tissue. This is consistent with the hypothesis that the peripheral lipoatrophy observed in HARS may result from selective regional changes in autonomic innervation [1,9].

How can this increased interstitial noradrenaline concentration in skeletal muscle and fat tissue be explained? Once noradrenaline is released from the nerve terminal into the synaptic cleft it can undergo reuptake into the neuron, or spill over from the synaptic cleft to the interstitium and further to the intravascular compartment. Although an increased interstitial noradrenaline concentration can be caused by an increased sympathetic firing rate, this was excluded by our findings. Alternatively, an increased interstitial noradrenaline concentration can be caused by a decrease in noradrenaline reuptake, even in the presence of a decreased sympathetic nerve firing rate to the skeletal muscle (as a result of negative feedback). Finally, even if neuronal noradrenaline reuptake is normal, intraneuronal noradrenaline metabolism by monoamine oxidase, which is located within the mitochondria may be inhibited and result in increased noradrenaline release into the synaptic cleft and subsequently in an increased interstitial noradrenaline concentration. It is tempting to speculate whether antiretroviral agents may affect either noradrenaline reuptake or monoamine oxidase activity, the latter for instance by way of nucleoside reverse transcriptase inhibitor-associated mitochondrial toxicity [10].

In summary, in the context of an unchanged global sympathetic activity, HIV-infected patients with HARS appear to have increased noradrenaline concentrations at the level of skeletal muscle and subcutaneous fat tissue, which may be consistent with the hypothesis that regional changes in autonomic activity contribute to the selective loss of peripheral fat as observed in HARS. These findings suggest that disturbances in local SNS activity play a role in this remarkable syndrome, but this requires further investigation.

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Departments of [a]Internal Medicine and [b]Chemical Endocrinology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; and Departments of [c]Internal Medicine, [d]Infectious Diseases, Tropical Medicine and AIDS, and [e]Endocrinology and Metabolism, Academic Medical Center of the University of Amsterdam, Amsterdam, the Netherlands.

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Advanced immunosuppression at entry to HIV care in the southeastern United States and associated risk factors

Cynthia L. Gay, Sonia Napravnik and Joseph J. Eron Jr

In this study we characterized factors associated with the late initiation of HIV care in the southeastern United States. At initiation of care, antiretroviral therapy was indicated for 75% of patients, 50% had a CD4 cell count of less than 200 cells/μl, and 27% presented with an AIDS-defining illness. Male sex was an independent predictor in multivariable analysis. These results indicate an urgent need to increase HIV testing for earlier diagnosis in the southeastern USA.

In the United States, over one-third of individuals develop an AIDS-defining illness within one year of HIV diagnosis, and an estimated 180 000–280 000 Americans are unaware of their HIV infection [1]. The initiation of antiretroviral therapy (ART) after an AIDS-defining illness or CD4 cell decline below 200 cells/μl increases the risk of morbidity and mortality [2,3]. In earlier research, male sex and older age were associated with an increased risk of late entry into HIV care in some [4–7] but not all studies [8–11]. Although the southeastern USA reports the greatest proportion of AIDS cases and deaths [12,13], no studies on the late initiation of HIV care have been conducted in this region. Therefore, we characterized entry to HIV care and the predictors of the late initiation of care with ART indicated.

The study population included patients initiating HIV care between 2000 and 2003 at the University of North Carolina HIV outpatient clinic, which is located in a large tertiary care facility. Clinical and demographic characteristics were abstracted from medical records. The indication for ART was defined as a CD4 cell count of less than 350 cells/μl, an HIV-RNA level greater than 100 000 copies/ml, or an AIDS-defining illness [14]. We considered a number of factors affecting entry to HIV care with ART indicated, including sex, age, race, insurance, distance-to-care, rural residence, HIV exposure group, alcohol and substance abuse, and major depressive disorder. Rural residence was defined as a metropolitan statistical area with a population of less than 50 000 [15].

We performed basic bivariate analyses and fit multivariable logistic regression models to identify characteristics predicting ART indication at entry to HIV care using the SAS statistical package (version 8.2; SAS Institute Inc., Cary, North Carolina, USA). The study was approved by the UNC Institutional Review Board.

Of 348 patients initiating HIV care during this period, 63% ($n = 220$) had not received any previous HIV care at any facility. Thirty-five per cent ($n = 77$) were women, and the median age was 37 years [interquartile range (IQR) 30, 45, Table 1]. Sixty-eight per cent ($n = 150$) initiated HIV care within one year of their first HIV-positive test, and 16% ($n = 35$) delayed care for more than 2 years. Among the 35 patients aware of their HIV diagnosis for more than 2 years, the median delay was 5 years (IQR 3, 10).

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Table 1. Initiation of HIV care with antiretroviral therapy indicated by patient characteristics.

Characteristic[a]	N	ART indicated %	Unadjusted odds ratio[b] (95% CI)	P value[c]	Adjusted odds ratio[d] (95% CI)	P value[e]
Sex						
Male	143	80	2.1 (1.1, 3.9)	0.02	2.8 (1.3, 6.2)	0.01
Female	77	66	Referent		Referent	
Race						
White	48	77	1.1 (0.5, 2.4)	0.77	1.4 (0.6, 3.2)	0.42
Other[f]	172	75	Referent		Referent	
Age (years)						
≤ 40	143	73	0.6 (0.3, 1.3)	0.20	0.8 (0.4, 1.7)	0.57
> 40	77	81	Referent		Referent	
Insurance						
Public or none	163	76	1.1 (0.6, 2.3)	0.72	1.5 (0.7, 3.2)	0.33
Private	57	74	Referent		Referent	
Residence (MSA)						
$\leq 50\,000$	79	81	1.6 (0.8, 3.2)	0.15	1.5 (0.7, 3.2)	0.32
$> 50\,000$	141	72	Referent		Referent	
Distance to clinic (miles)						
≤ 60	124	74	0.9 (0.5, 1.6)	0.62	1.0 (0.5, 1.9)	0.95
> 60	96	77	Referent		Referent	
MSM						
Yes	53	72	0.8 (0.4, 1.6)	0.47	0.4 (0.2, 1.1)	0.08
No	167	77	Referent		Referent	
Injection drug use						
Yes	23	78	1.2 (0.4, 3.4)	0.74	0.9 (0.3, 3.1)	0.90
No	197	75	Referent		Referent	
Alcohol abuse						
Yes	62	85	2.3 (1.1, 5.1)	0.03	2.4 (1.0, 6.1)	0.06
No	158	72	Referent		Referent	
Substance abuse						
Yes	72	76	1.1 (0.6, 2.1)	0.82	0.7 (0.3, 1.6)	0.42
No	148	75	Referent		Referent	
Major depression						
Yes	128	74	0.9 (0.5, 1.6)	0.62	0.8 (0.4, 1.6)	0.58
No	92	77	Referent		Referent	

ART, Antiretroviral therapy; CI, confidence interval; MSA, metropolitan statistic area; MSM, men who have sex with men.
[a]MSM and injection drug use are categories of mode of HIV exposure and are not mutually exclusive.
[b]Unadjusted odds ratios were not adjusted for any other characteristic.
[c]*P* values were calculated using Pearson's chi-square test.
[d]Adjusted odds ratios were based on a full multivariable logistic regression model including sex, race, age, insurance, residence, distance to clinic, MSM, injection drug use, alcohol abuse, substance abuse and major depression.
[e]*P* values were based on the likelihood ratio test from the full multivariable logistic regression model.
[f]Other race category includes: 87% African American ($n = 149$); 10% Hispanic ($n = 18$); 2% American Indian/Alaska native ($n = 3$); and 1% Asian/Pacific Islander ($n = 2$).

At entry to care, 29% ($n = 64$), 21% ($n = 46$), 20% ($n = 45$), and 30% ($n = 65$) had a CD4 cell count of less than 50, 50–199, 200–349 and greater than 349 cells/μl, respectively. The median HIV-RNA level was 4.8 $\log_{10}$ copies/ml (IQR 4.1, 5.3), and 46% ($n = 101$) had HIV-RNA levels greater than 100 000 copies/ml. Twenty-seven per cent ($n = 59$) presented with an AIDS-defining illness, most commonly, *Pneumocystis jiroveci* pneumonia (39%), esophageal candidiasis (16%), and extrapulmonary cryptococcosis (14%). Sixteen per cent ($n = 35$) were referred directly from an inpatient unit, and 11% ($n = 24$) were diagnosed with HIV during this index hospitalization.

On initial presentation, ART was indicated for 75% of patients ($n = 165$) based on the CD4 cell count, HIV-RNA level, and/or an AIDS clinical condition, and for 71% ($n = 156$) it was based solely on the CD4 cell count. ART was indicated for 78% (117/150), 57% (20/35), and 83% (29/35) of patients entering HIV care one year or less, 1–2 years, and over 2 years from HIV diagnosis, respectively ($P = 0.02$). In bivariate analyses, male sex ($P = 0.02$) and alcohol abuse ($P = 0.03$) predicted an indication for ART at presentation (Table 1). Male sex remained a statistically significant independent predictor in the multivariable model. Notably, race, rural residence and distance to clinic were not predictive.

This study demonstrates that most HIV-infected patients in the southeastern USA initiate HIV care with advanced immunosuppression, and the majority with ART indicated [14]. The degree of immunological impairment in patients initiating care at our facility is among the highest reported [4–9,11,16–19]. Male sex independently predicted ART indication at the first visit, even after the exclusion of pregnant women ($n = 10$). These findings have been observed by others [4–7], and indicate that targeted interventions are needed among men to increase earlier

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HIV diagnosis. Our alcohol use findings warrant further attention, given the high rates of alcohol abuse among HIV-infected patients [20,21], particularly in the rural southeastern USA, where treatment is limited.

This is the first investigation of HIV care initiation by the place of residence, despite the fact that rural areas in the southeastern USA have experienced the largest increases in AIDS cases [12,22–24]. Rural residence was not statistically associated with the late initiation of HIV care in this study. However, the predominantly rural and semi-rural nature of the southeastern USA may contribute to an overall observed substantial delay in accessing HIV care, given the scarce medical, social, and HIV testing services, limited transportation, poverty, decreased perception of risk, and possibly greater perceived stigma and confidentiality concerns [25,26].

We noted substantial numbers of individuals with high HIV-RNA levels at entry to care. High HIV-RNA levels have been associated with an accelerated onset of AIDS-related illness and CD4 cell decline [27], and an increased risk of transmitting HIV to sexual partners [28,29]. The high HIV-RNA levels in our patients highlight missed opportunities for treatment and the prevention of further HIV transmission. Most patients were HIV diagnosed within 2 years of HIV care initiation, indicating that late entry was not related to the delay from testing to accessing care.

Our study only included patients initiating care at a single center and may not be generalizable to other populations. We were also limited by our inability to assess the effect of factors such as lack of social support, fear of discrimination or stigmatization, lack of general HIV knowledge, or low perception of HIV risk on late entry to HIV care, although these may be especially salient to HIV testing and medical care initiation.

Overall, this study indicates that patients in the southeastern USA initiate HIV care with ART indicated, reflecting advanced immunosuppression and a need for care. As ART benefits diminish with late therapy initiation, earlier access to HIV care would probably improve morbidity and mortality in this population. A prolonged interval between HIV acquisition, diagnosis, and access to care, delays transmission–reduction measures and secondary prevention strategies. Our results indicate an urgent need to increase earlier HIV diagnosis and linkage to care, especially in the southeastern USA.

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Successful use of genotypic resistance testing in HIV-1-infected individuals with detectable viraemia between 50 and 1000 copies/ml

Laura Waters, S. Mandalia and David Asboe

Traditionally an HIV viral load of over 1000 copies/ml was presumed necessary for genotypic resistance testing. We performed a retrospective analysis to assess rates of viral amplification on viral loads between 50 and 1000 copies/ml. Amplification was significantly more likely for 200–1000 copies/ml than 50–200 copies/ml; most samples amplified successfully, supporting the use of genotyping for low-level viraemia.

In the era of HAART viral load monitoring is an integral part of therapeutic monitoring in developed countries. Virological suppression below 20–50 copies/ml is associated with a more durable virological response than suppression to 50–400 copies/ml [1–3].

The early diagnosis of virological failure is essential, because an important cause is the development of genotypic mutations in the reverse transcriptase or protease genes that confer resistance to antiretroviral agents. Genotypic testing is used to detect which mutations have developed and select the subsequent antiviral regimen accordingly. Historically, a lower cut-off of 1000 copies/ml had been used to determine when a genotype can be performed; this was based on physician experience and anecdotal evidence.

At our centre resistance tests are routinely reviewed in a weekly ‘virtual clinic’, and it was noticed that a number of tests had been requested on samples with a viral load below 1000 copies/ml, but the sample had been successfully amplified for genotyping. We therefore reviewed all resistance assays performed in samples with an HIV viral load of less than 1000 copies/ml.

We have a large, prospectively collected database including demographic data, treatment history, clinical events and the results of laboratory investigations, including viral load assays and resistance tests. Using this database, we identified all individuals between July 2001 and July 2003 with at least two consecutive viral loads above 50 but less than 1000 copies/ml who underwent genotyping in the same period. For the resistance assays, HIV-1 protease–reverse transcriptase sequences were generated on an ABI 3730XL sequencer using Virco BVBA (Mechelen, Belgium) proprietary primer mixers. Viral loads at the time of resistance testing were stratified into ranges and chi-squared testing was used to analyse the differences in rates of successful genotyping within those ranges.

A total of 112 genotype tests were attempted on individuals with two consecutive viral loads in the 50–1000 copies/ml range. The likelihood of the successful amplification of genetic material and sequencing is demonstrated in Table 1. Overall, 73% of samples were clade B and 27% were non-B.

The likelihood of successful genotyping was significantly greater for a viral load between 200 and 1000 copies/ml compared with 50 and 200 copies/ml ($P = 0.009$).

Table 1. Likelihood of the successful amplification of genetic material and sequencing.

Viral load range (copies/ml)	No. of samples	% Successfully amplified (95% CI)
50–200	36	69.4 (51.9–83.6)
200–600	49	90.2 (77.8–96.6)
600–1000	27	92.9 (75.7–99.1)

CI, Confidence interval.

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In summary, we demonstrate a significant chance of successful genotypic analysis on samples with an HIV viral load less than 1000 copies/ml. In particular, for samples with a viral load between 200 and 1000 copies/ml there was a greater than 90% chance of successful amplification. Even for samples in the 50–200 copies/ml range there was an almost 70% chance of successful genotyping. We recommend genotypic testing on all individuals with a viral load in excess of 200 copies/ml and also for individuals with persistent viraemia below 200 copies/ml.

Department of Genitourinary and HIV Medicine, St Stephens Centre, Chelsea and Westminster Hospital, London, UK.

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Point of care testing for antiretroviral therapy-related lactic acidosis in resource-poor settings

Louise C. Ivers and Joia S. Mukherjee

Lactic acidosis is a rare but potentially life-threatening complication of antiretroviral therapy (ART) and is commonly considered in the differential diagnosis of patients on ART. In the developing world, definitive diagnosis by laboratory measurement of lactate may be impossible. Point-of-care devices are available that provide simple, accurate measurements of lactic acid levels at relatively low cost. Their use in an HIV treatment programme in rural Haiti has greatly assisted clinical decision-making in patients with symptoms suggestive of lactic acidosis.

As HIV treatment is scaled up in resource-poor settings, we must consider how best to diagnose and manage the side-effects of antiretroviral medications in the setting of minimal laboratory facilities. Rather than use this challenge as another obstacle to the scale-up of HIV treatment worldwide, we must look to innovative ways to use existing technologies.

Lactic acidosis is an uncommon, but sometimes fatal complication of antiretroviral therapy (ART). Clinical criteria alone are insufficient for diagnosis because the disorder is associated with non-specific symptoms such as nausea and vomiting. Definitive diagnosis includes the finding of elevated lactic acid levels in the plasma.

In the developing world, gastrointestinal complaints are particularly common and the diagnosis of lactic acidosis may frequently be considered in patients on ART. The inability to exclude lactic acidosis with confidence because of the lack of specialized laboratory equipment often leads to diagnostic uncertainty, and at times the unnecessary interruption of ART. Lack of confirmation of the diagnosis is of particular concern in settings in which alternative ART regimens may not be available for patients who stop their first-line regimes.

Concerned with the possibility of lactic acidosis in our patients receiving combination ART in rural Haiti, we searched for alternatives to the standard laboratory evaluation for plasma lactic acid levels.

Point-of-care testing is used frequently in medical care and is instrumental for example, in the care of diabetic patients. Devices are available, however, for a range of examinations beyond glucose, including a number for measuring lactic acid. In February 2005, we piloted the use of a hand-held, point-of-care testing device for measuring lactic acid on whole blood obtained by finger stick in our clinics in rural Haiti (Accutrend lactate portable lactate analyser; Sports Resource Group, Inc. USA). The device provides lactate results within 60 seconds of placing a drop of whole blood on the test strip and is powered by three 1.5-volt batteries. The measuring range for whole blood is 0.8–22 mmol/l and for plasma it is 0.7–27 mmol/l [1].

Point-of-care devices for lactate have been evaluated in intensive care units and trauma centers, and were previously validated as effective methods to detect blood or plasma lactate levels, correlating well with standard laboratory measurements [2,3]. Eliminating the need for centrifugation, specialized laboratory equipment, requiring a very small quantity of blood, and eliminating the need to transport blood specimens on ice, point-of-care devices provide a significant advantage in the measurement of lactic acid.

These advantages make the devices particularly well suited to use in resource-poor settings, where they can assist in important clinical decision-making for patients with suggestive symptoms. After a short training session, the devices have been well received and are in use by our staff in Haiti.

Although knowledge of the lactate level is only the first step in the management of possible antiretroviral-related

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lactic acidosis, it is an extremely useful measurement. As we scale up HIV care worldwide, we must look to use existing technologies in novel settings if we are to combat the epidemic effectively.

Partners In Health, Boston, USA; and Department of Medicine, Divisions of Social Medicine and Health Inequalities, and Infectious Diseases, Brigham and Womens Hospital and Harvard Medical School, Boston, USA.

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Tracing the origin of Brazilian HTLV-1 as determined by analysis of host and viral genes

Luiz C.J. Alcantara[a,b], Tulio de Oliveira[d], Michelle Gordon[c], Oliver Pybus[d], Rita Elizabeth Mascarenhas[a,b], Magda O. Seixas[e], Marilda Gonçalves[e], Carol Hlela[f], Sharon Cassol[f] and Bernardo Galvão-Castro[a,b]

We compared the genetic diversity of the Brazilian human T-cell lymphotropic virus type 1 isolates with those found in KwaZulu–Natal (KZN), South Africa, and with the genetic background of the hosts. The seroprevalence rate in KZN was 1.7%. All sequences belonged to the A subgroup. The presence of South African sequences in two different clusters from Brazil, and the finding of the β^A-globin haplotype in infected hosts are consistent with the transmission of this virus from southern Africa to Brazil.

The origins of human T-cell lymphotropic virus type 1 (HTLV-1) in Salvador, a Brazilian city in the Bahia State, are not fully understood and are difficult to trace. One hypothesis suggests that the virus was introduced into south America from Africa, during the post-Columbian slave trade [1]. The majority of Africans who came to Salvador during this period of time were from west Africa, where only the HTLV-1 C subgroup of the Cosmopolitan (a) subtype has been found. Previous studies reported that HTLV-1 strains from Salvador belong to the A subgroup [2], as it was also previously demonstrated in KwaZulu–Natal (KZN), South Africa [3]. We attempted to resolve this discrepancy by studying the β^A-globin haplotype of HTLV-1-infected individuals living in Salvador [2]. The detection of HTLV-1a subgroup A among the Bantu people from Salvador could suggest that Brazil strains may have originated from southern Africa. To examine this possibility, we conducted detailed sequence and evolutionary analyses, comparing the genetic diversity and molecular phylogenies between Brazil and KZN HTLV-1 isolates, and with the genetic background of the infected hosts.

A total of 1435 samples were collected from HIV-1-uninfected and infected treatment-naive individuals obtained in Durban, KZN, South Africa, and surrounding areas, after approval from the University of KZN Ethical Board. Plasma were screened for HTLV-1/2 antibodies by enzyme immunoassay. DNA was extracted from peripheral blood mononuclear cells (enzyme immunoassay-positive samples) using QIAamp (Qiagen, USA). *Pol* gene-nested polymerase chain reaction was performed to differentiate between HTLV-1 and 2 [4]. Long-term repeat (LTR) fragments were amplified from 29 South Africa DNA samples and from 10 samples from Salvador collected in a previous study [2]. The products were purified and sequenced directly on a 3100 genetic analyser (Applied Biosystems, California, USA). Phylogenetic trees of 724 basepair LTR sequences were generated using the neighbour-joining and maximum-likelihood (ML) methods of PAUP* software, version 4.0b10 [5]. Two sets of LTR sequence alignments from mother–infant pairs in KZN were available for an estimation of the HTLV-1 evolutionary rate (nucleotide/site/year). The evolutionary rate of each set was calculated using a homogeneous Poisson model, as previously described [6]. Genotyping of human β^A-globin was performed on 10 HTLV-1-infected individuals (five South Africa and five Brazil) as previously described [4]. The haplotype patterns for South African and Brazil isolates were compared with those typical haplotypes from the Central African Republic (CAR; Bantu), Benin, Senegal and Cameroon.

The HTLV-1 seroprevalence in KZN was 1.7% (24). The average intersequence diversity among Brazil LTR sequences was significantly higher than among KZN sequences (1.42 × 0.7%), even when epidemiologically linked samples were excluded from the KZN analysis (0.78%). As expected, sequences from transmission pairs were highly conserved (divergence of 0.1%) with only one polymorphism being detected in the LTR region of one family. Phylogenetic analysis showed that all South Africa and Brazil sequences belonged to subgroup A of the HTLV-1a (Fig. 1). Two distinct clusters of Latin American sequences (A and B) were identified within the A subgroup, both supported by high bootstrap and by ML. At the main cluster (A), two new isolates from KZN (HTLV04 and HTLV06) formed a monophyletic outgroup, a finding also supported by both bootstrap and ML. The second cluster (B) contained a new KZN isolate

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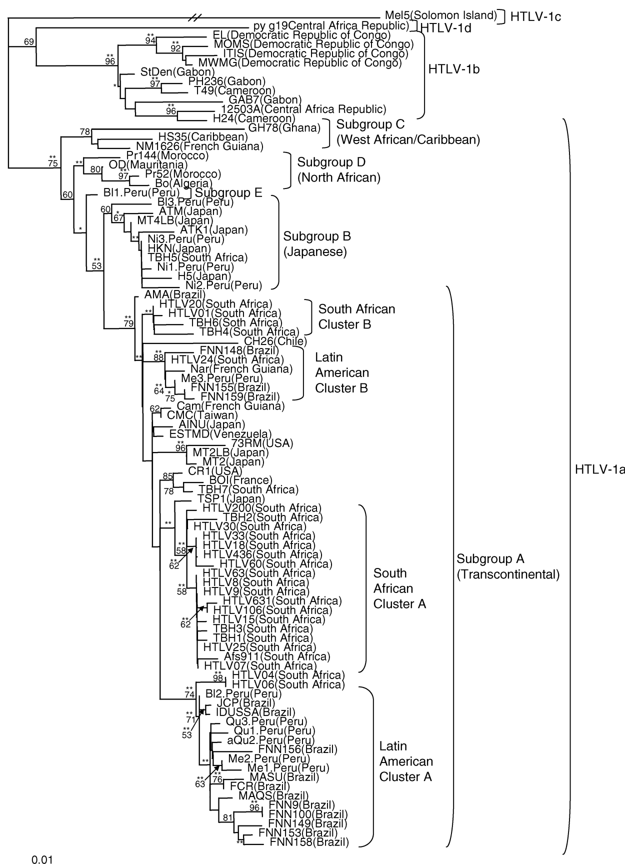

Fig. 1. Rooted neighbour-joining tree of human T-cell lymphotropic virus type 1 strains based upon a 724 bp of the total long-term repeat region. The Tamura-Nei evolutionary model with (-distribution was selected and the tree was drawn using TreeView, version 1.4. The bootstrap values (above 50% and using 1000 bootstraps) on the branches represent the percentage of trees for which the sequences at the right end of the branch form a monophyletic group. Mel5 is used as the outgroup. Geographical origin and ethnic origin are given between parentheses. Newly sequenced long-term repeats included in this analysis (in bold) are the following Salvador and South African isolates. The ** means that the maximum-likelihood method was highly significant with a *P* value of less than 0.001 or significant with a *P* value of less than 0.005. The GenBank accession numbers of the new strains are DQ005546–DQ005574.

(HTLV24). Analysing all 20 β^A-globin chromosomes, we identified two Senegal, six Benin and 12 CAR haplotypes. Among South Africa, four were homozygous for either the Benin/Benin ($n = 2$) or CAR/CAR ($n = 2$) haplotypes; and one was Benin/CAR heterozygote. Two Brazil were homozygous (CAR/CAR) and three (60%) were heterozygous: two for the Senegal/CAR, and one for the Benin/CAR haplotype. Based on a single mutation in the LTR region of one mother–child pair, the minimum and maximum age of transmission was calculated to be 20 and 102 years. The average HTLV-1 evolutionary rate (756 sites) from both transmission pairs was estimated to be 2.16×10^{-5}, with the upper and lower 95% intervals estimated to be 1.30×10^{-4} and 1.13×10^{-6}, respectively. To increase the statistical power of our analysis, we increased our sample size, including published evolutionary data from an additional 16 transmission chains [6]. The combined datasets contained three LTR mutations, and resulted in an average evolutionary rate of 4.49×10^{-6}, with lower and higher intervals in the range of 1.08×10^{-6} and 1.34×10^{-5}. The total transmission time (t) for the collective dataset (18 transmission pairs) was calculated to be between 539 and 1203 years.

The high degree of relatedness is consistent with the transmission of HTLV-1a subgroup A from South Africa to Brazil, presumably during the slave trade process. Moreover, three LTR sequences from South Africa actually segregated within the Brazil clusters, suggesting that there were probably multiple introductions of HTLV-1 from South Africa to Brazil. Transmission between South Africa and Brazil is also consistent with the similar prevalence rates in KZN (1.7%) and Salvador (1.76%), a city where more than 80% of the population is of African origin [2,7]. The finding that all HTLV-1-infected individuals in our study had the same β^A-globin haplotype is also consistent with the transmission from southern Africa. The source of the CAR haplotype in South Africa is not known, but may reflect the migration of the Bantu population from north to South Africa during the last 3000 years, an event that gave origin to the Zulu tribes of South Africa. Alternatively, the Benin haplotype may have been introduced recently (during the past 300 years) [8]. Finally, the low level of diversity observed among South African isolates was supported by evolutionary analysis of two mother-to-child transmission chains, when the attempts to improve the accuracy of this value, by including data from 16 previously published transmission chains [6], resulted in an estimated evolutionary rate of 4.49×10^{-6}. Unlike the HIV-1, HTLV-1 shows little evidence of adaptation or natural selection, exhibiting a low evolutionary rate. As a result, spatial and demographic processes, such as multiple introductions of the virus during the slave trade, are likely to be among the main processes shaping the structure of phylogenetic trees. Understanding the differences between HTLV-1 and HIV-1 may lead to new insights for controlling the spread and genetic evolution of these important human pathogens.

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[a]*Public Health Advanced Laboratory, Gonçalo Moniz Research Center, Oswaldo Cruz Foundation, Salvador, Bahia, Brazil;* [b]*Bahia School of Medicine and Public*

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Health/Foundation for Science Development, Salvador, Bahia, Brazil; [c]Molecular Virology and Bioinformatics Unit at Africa Centre for Health and Population Studies, Nelson Mandela Medical School, University of KwaZulu–Natal, Durban, South Africa; [d]Department of Zoology, University of Oxford, Oxford, UK; [e]Molecular Biology and Pathology Laboratory, Gonçalo Moniz Research Center, Oswaldo Cruz Foundation, Salvador, Bahia, Brazil; and [f]HIV-1 Immunopathogenesis Unit, Department of Virology, University of Pretoria, Pretoria, South Africa.

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