



## Screening and treating cervical cancer in HIV-positive women in Cambodia.

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## Role of HLA Class I (HLA-A, B) and HLA Class II (HLA-DRB, DQB) in HIV-1 Patients With and Without Pulmonary Tuberculosis

AIDS caused by HIV infection is endemic all over the globe and it is on the rise especially in resource-limited countries. Over 33 million people are living with HIV, 2.5 million are newly infected, and 2.1 million people are died of AIDS.<sup>1</sup> Individuals with impaired cell-mediated immunity due to AIDS have a greatly increased risk of coinfection with *Mycobacterium tuberculosis* (MTB).<sup>2</sup> The coinfection of HIV-1 and MTB causes 2 infectious diseases endangering human health significantly. The pathogenesis of HIV-1 and PTB coinfection is available. The factors influencing the greater interindividual variability to susceptibility to PTB coinfection and progression of AIDS are not known. This may be due to considerable varied immune responses of HIV-1 and MTB exposed individuals may result from the different genetic background. Major histocompatibility complex class I-restricted CD8<sup>+</sup> T cells are important for the generation of protective immune response in MTB infection. CD8<sup>+</sup> CTL (cytotoxic T lymphocytes)-derived IFN- $\gamma$  may be especially important both for cells lacking major histocompatibility complex class II molecules.

Both HLA class I and class II genes have been shown to be associated with susceptibility or resistance to HIV infection.<sup>3</sup> Among the HLA class II alleles, HLA-DQB1 and HLA-DBP1 alleles have been shown to be associated with HIV infection.<sup>4,5</sup> But, we have much less information about the HLA-linked genetic control of susceptibility to HIV-1 and MTB coinfection. We have attempted to study the role of HLA class I (HLA-A, B) and HLA class II (HLA-DRB, DQB) in HIV-1 patients with or without pulmonary tuberculosis (PTB).

A total of 390 individuals comprising 102 HIV-1+ patients coinfecting with PTB, 88 HIV-1+ patients without PTB, and 200 healthy controls were included in HLA class I analysis. And a total of 184 individuals comprising 30 HIV-1+ patients coinfecting with PTB, 25 HIV-1+ patients without PTB, and 129 healthy controls were included in HLA class II analysis. The clinically confirmed cases of HIV-1 with or without PTB were included in our study. Class I antigens (HLA-A, B) were typed serologically by microlymphocytotoxicity assay. HLA class II (HLA-DRB, DQB) typing was done molecularly by PCR-SSOP (polymerase chain reaction-sequence specific oligonucleotide probing) method using kit (DynaL Kit; Invitrogen, Carlsbad, CA). The frequencies of the HLA class I (HLA-A, B) and HLA class II (HLA-DRB, DQB) alleles were determined by direct allelic count and expressed as percent frequency using standard software.

The HLA class I and HLA class II alleles identified among HIV+/PTB+ coinfecting patients compared with HIV+ PTB- patients and healthy controls are given in Table 1. Significantly increased frequency of HLA-B8 was observed in HIV+/PTB+ coinfecting patients when compared with healthy controls [ $P = 0.011$ , odds ratio (OR): 3.335, 95% confidence interval (CI): 1.35 to 8.18] but decreased significantly in HIV+ PTB- patients ( $P = 0.086$ , OR: 0.502, 95% CI: 0.24 to 1.03). Likewise, HLA-DQB1\*030103 was significantly increased in HIV+/PTB+ coinfecting patients as against healthy controls ( $P < 0.0001$ , OR: 107.5, 95% CI: 6.195 to 1865.3). Similarly, HLA-DQB\*060102 allele frequency was observed in HIV+/PTB+ coinfecting patients as against healthy controls ( $P = 0.003$ , OR: 4.808, 95% CI: 1.72 to 13.39) and also when compared with HIV+ PTB- patients ( $P = 0.0207$ , OR: 16.352, 95% CI: 0.91 to 290.99). A significantly increased frequency of HLA-A2 ( $P = 0.015$ , OR: 1.762, 95% CI: 1.13 to 2.73), HLA-B17 ( $P = 0.017$ , OR: 1.973, 95% CI: 1.15 to 3.37), HLA-B22 ( $P = 0.029$ , OR: 2.606, 95% CI: 1.16 to 5.85), HLA-DRB1\*040301 ( $P = 0.006$ , OR: 7.727, 95% CI: 1.79 to 33.3), HLA-DRB1\*090102 ( $P = 0.012$ , OR: 9.143, 95% CI: 1.63 to 51.174), HLA-

DRB1\*140103 ( $P = 0.024$ , OR: 13.526, 95% CI: 1.381 to 132.49), and HLA-DQB1\*050201 ( $P < 0.0001$ , OR: 28.556, 95% CI: 8.36 to 242.16) and a significantly decreased frequency was observed in HLA-B5 ( $P = 0.009$ , OR: 0.434, 95% CI: 0.236 to 0.799), HLA-DQB1\*030101 ( $P = 0.045$ , OR: 0.219, 95% CI: 0.051 to 0.940), HLA-DQB1\*060101 ( $P = 0.012$ , OR: 0.334, 95% CI: 0.145 to 0.770), alleles in HIV+/PTB+ coinfecting patients when compared with healthy controls. Significantly increased frequency of HLA-A10 ( $P = 0.086$ , OR: 0.502, 95% CI: 0.24 to 1.03), was observed in HIV+/PTB+ coinfecting patients when compared with HIV+ PTB- patients.

We have studied, HLA-A, B, DRB, and DQB loci to find out the role of these HLA alleles in HIV+/PTB+ coinfection. Significantly increased frequency of HLA-B8 and HLA-DQB\*030103 in HIV+/PTB+ coinfecting patients against controls may suggest that these alleles play an associative role in HIV infection and PTB development. But on the contrary, a significantly decreased frequency of HLA-B8 and HLA-DQB\*030103 observed in HIV+/PTB+ coinfecting patients when compared with HIV+ PTB- patients may be suggestive of their protective role in PTB development. Our study reveals that HLA-B8 and HLA-DQB\*030103 are associative to enhance HIV infection but play a protective role in development of PTB coinfection. The decreased frequency of HLA-DQB1\*0301 has been reported in PTB patients from China.<sup>6</sup> In our study, HLA-DQB1\*030101 is decreased in HIV+/PTB+ coinfecting patients compared with controls suggesting that it may play a protective role in HIV+/PTB+ coinfection, whereas HLA-DQB\*030103 is associative in HIV infection but plays a protective role in development of PTB in HIV infected patients.

Recently among south Indians, an increased frequency of HLA-DQB1\*0601 has been reported in HIV- PTB+ and HIV+ PTB+ patients, suggesting that HLA-DQB1\*0601 is associated with susceptibility to PTB as well as development of PTB in HIV patients.<sup>7</sup> Further earlier association of HLA-DQB1\*0601 with susceptibility to PTB has also been reported in south India.<sup>8</sup> In contrast to the

**TABLE 1.** Significant HLA Alleles Identified Among the TB Coinfected HIV Patients from Western India

HIV+ PTB+ (n = 102) vs Controls (n = 200) ↑	HIV+ PTB+ (n = 102) vs Controls (n = 200) ↓	HIV+ PTB+ (n = 102) vs HIV+ PTB- (n = 88) ↑	HIV+ PTB+(n = 102) vs HIV+ PTB- (n = 88) ↓
HLA-B8	—	—	HLA-B8
—	HLA-B5	—	—
HLA-B22	—	—	HLA-A10
HLA-B17	—	—	—
HLA-A2	—	—	—
HIV+ PTB+ (n = 30) vs Controls (n = 129) ↑	HIV+ PTB+ (n = 30) vs Controls (n = 129) ↓	HIV+ PTB+ (n = 30) vs HIV+ PTB- (n = 25) ↑	HIV+ PTB+(n = 30) vs HIV+ PTB- (n = 25) ↓
HLA-DRB1*040301	HLA-DQB1*030101	—	—
HLA-DRB1*090102	HLA-DQB1*060101	—	—
HLA-DRB1*140103	—	—	—
HLA-DQB1*030103	—	—	HLA-DQB1*030103
HLA-DQB1*060102	—	HLA-DQB1*060102	—
HLA-DQB1*050201	—	—	—

above 2 studies on south Indian population, it is reported that HLA-DQB1\*0601 plays a protective role against HIV disease progression in Europeans.<sup>9</sup> In our study, a significantly increased frequency of HLA-DQB\*060102 in HIV+/PTB+ coinfecting patients when compared with controls and HIV+ PTB- patients may suggest its strong association with both HIV infection and PTB coinfection. But on the contrary, HLA-DRB1\*060101 frequency was significantly decreased in HIV+/PTB+ coinfecting patients compared with controls, thereby may protect from HIV infection and PTB development. HLA-DQB1\*0502 allele is reportedly related to high risk of developing TB in population from Asia and Latin America.<sup>10</sup> In the present study, frequency of HLA-DQB1\*050201 is increased in HIV+/PTB+ coinfecting patients compared with healthy subjects to show that HLA-DQB1\*050201 may be associated with HIV+/PTB+ coinfection. There was no considerable change in frequency of HLA-A10 in HIV+/PTB+ coinfecting patients when compared with controls and also decreased frequency was observed when compared with HIV+ PTB- patients. This suggests that HLA-A10 may not be associated with HIV infection and also may play a protective role in PTB development. HLA-A31 and HLA-B41 antigens and the HLA-DRB1\*10 and HLA-DQB1\*05 were overrepresented in Brazilian patients with AIDS and tuberculosis (TB), suggesting association to TB with AIDS.<sup>11</sup> As reported earlier, HLA-DRB1\*13 is associated with susceptibility to HIV-1 infection, whereas HLA-

DQB1\*0203 and DRB1\*01 are resistant to HIV-1 infection, which may vary in different ethnic groups.<sup>4</sup> HLA-DRB1\*15 is susceptible in PTB development and DRB1\*11 may be protective allele in Chinese population.<sup>6</sup> In the present study, HLA-DRB alleles HLA-DRB1\*040301, DRB1\*090102, DRB1\*140103 and also HLA-DQB1\*050201 allele are significantly increased in HIV+/PTB+ coinfecting patients compared with healthy controls. Thus, these alleles may be associated to susceptibility of HIV+/PTB+ coinfection among Indians.

In conclusion, different HLA alleles may render susceptibility or protection to an infection in different ethnic population. Both HLA class I and class II alleles may influence immunopathogenesis of either HIV and/or PTB infection. Further study on the HIV progression and resistant TB would enlighten the mechanism of action of the HLA in HIV and/or PTB infection.

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## CD8<sup>+</sup> Hyperactivation and Senescence Correlate With Early Carotid Intima–Media Thickness in HIV+ Patients With No Cardiovascular Disease

### To the Editor:

HIV-infected patients are at increased risk for atherosclerosis and cardiovascular disease (CVD).<sup>1</sup> By assessing atherosclerosis, carotid artery intima–media thickness (IMT) also proved a predictor of future vascular events.<sup>2</sup> HIV-positive patients display higher carotid IMT and progression compared with healthy controls.<sup>3</sup> Besides conventional risk factors, atherosclerosis and IMT increases in HIV-positive patients may be hastened by immune and inflammatory pathways,<sup>4,5</sup> given T-lymphocyte hyperactivation in HIV/AIDS.<sup>6</sup>

Given these premises, we cross sectionally investigated the possible correlations between CD8<sup>+</sup> activation/maturation profiles and early carotid IMT increase in 64 HIV-infected patients: 17 experienced CVD within the past 2 years, whereas 47 patients never experienced CVD. This latter group of patients underwent carotid artery Doppler ultrasonography (ESAOTE power color-Doppler, 7.5 MHz probes) and were divided in the following: increased IMT patients (IIMT, n = 19) if either left and/or right carotid IMT  $\geq$  1 mm and normal IMT (NIMT, n = 28) if both left and right carotid IMT < 1 mm.

Figure 1A shows the characteristics of patients under study. Aiming to

assess patients' inflammatory profile, we quantified plasma proinflammatory cytokines, tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), and interleukin-6 (IL-6) in a subgroup of 25 unselected subjects (R&D, Abingdon, UK). As expected, CVD patients (n = 6) presented higher TNF- $\alpha$  levels reaching significance vs NIMT (n = 10), whereas no differences were shown between CVD and IIMT (n = 9) (CVD:  $3.39 \pm 0.5$ ; IIMT:  $2.79 \pm 0.6$ ; NIMT:  $2.2 \pm 0.35$ ,  $P = 0.04$  and  $0.56$  for CVD vs NIMT and IIMT, respectively). Interestingly, IIMT subjects displayed a tendency to more elevated TNF- $\alpha$  compared with NIMT ( $P = 0.24$ ). No differences were shown in plasma IL-6 among groups (Fig. 1B). Having shown a similar proinflammatory cytokine pattern in IIMT and CVD patients, we detailed CD8<sup>+</sup> phenotype by flow cytometry: CD8/CD38, CD8/CD95, CD8/127, and CD8/CD38/45R0 (Coulter ESP; Beckman Coulter, Milano, Italy). As compared with IIMT and NIMT as a whole, CVD patients displayed significantly higher mean activated CD38<sup>+</sup> CD8<sup>+</sup> (CVD:  $2.8\% \pm 0.65\%$ ; IIMT and NIMT:  $1.4\% \pm 0.2\%$ ,  $P = 0.03$ ) (Fig. 1C). Interestingly, when confronted individually, IIMT displayed significantly higher CD38<sup>+</sup> CD8<sup>+</sup> than NIMT, whereas no differences were shown between IIMT and CVD patients (IIMT:  $2.1\% \pm 0.4\%$ ; NIMT:  $1\% \pm 0.21\%$ ,  $P = 0.005$  for IIMT vs NIMT and CVD vs NIMT;  $P = 0.58$  for CVD vs IIMT) (Fig. 1C). Furthermore, when confronted to NIMT, IIMT patients showed a nonsignificant trend to lower CD8<sup>+</sup> expression of the death receptor Fas (CD95<sup>+</sup>) (IIMT:  $2.3\% \pm 0.54\%$ ; NIMT:  $3\% \pm 0.65\%$ ,  $P = 0.2$ ), suggesting a possible CD95 internalization on apoptosis-committed cells<sup>7</sup> (Fig. 1C) and significantly lower mean central memory CD127<sup>+</sup> CD8<sup>+</sup> (IIMT:  $11.7\% \pm 0.79\%$ ; NIMT:  $14.8 \pm 0.98\%$ ,  $P = 0.04$ ) (Fig. 1D). No differences were detected between IIMT and CVD in both parameters (CVD: CD95<sup>+</sup> CD8<sup>+</sup>:  $2.67\% \pm 0.73\%$ ,  $P = 0.9$ ; CD127<sup>+</sup> CD8<sup>+</sup>:  $14.3\% \pm 1.46\%$ ,  $P = 0.2$ ) (Figs. 1C, D). Similar proportions of terminally differentiated CD38<sup>+</sup> 45R0<sup>+</sup> CD8<sup>+</sup> were measured among groups (CVD:  $10.93\% \pm 2.9\%$ ; IIMT:  $9.1\% \pm 1.1\%$ ; NIMT:  $9.4 \pm 0.99$ ,  $P > 0.05$  for each pairwise comparison) (Fig. 1D). We further investigated the possible association between immune parameters, IMT

and clinical–metabolic parameters. Considering all patients as a whole, activated CD38<sup>+</sup> CD8<sup>+</sup> subset correlated positively with waist circumference ( $R = 0.35$ ,  $P = 0.016$ ) and carotid IMT ( $R = 0.5$ ,  $P = 0.001$ ) and negatively with CD127<sup>+</sup> CD8<sup>+</sup> ( $R = -0.29$ ,  $P = 0.05$ ). When analyzed separately, a trend to a significant positive association was yielded only in IIMT between IMT and CD38<sup>+</sup> CD8<sup>+</sup> ( $R = 0.49$ ,  $P = 0.067$ ), whereas no correlation was shown in NIMT.

Our data suggest that early IMT in HIV-infected patients is associated to a hyperactivated CD8<sup>+</sup> phenotype analogous to CVD subjects, allowing to hypothesize an association between pathologic IMT and generalized CD8<sup>+</sup> activation. By showing a contraction of CD95-expressing and central memory CD127<sup>+</sup> CD8<sup>+</sup> T cells despite comparable primed/activated CD38<sup>+</sup> 45R0<sup>+</sup> CD8<sup>+</sup>,<sup>8,9</sup> our data suggest a likewise senescent CD8<sup>+</sup> pool with greater apoptotic history and disrupted maturation as consequences of immune hyperactivation on CD8<sup>+</sup> pool in patients with early atherosclerosis and subjects with overt CVD. By delineating a specific CD8<sup>+</sup> phenotype in patients with heightened IMT, our data advocate the thorough investigation of immunological parameters as adjuvant markers of cardiovascular risk in HIV/AIDS.

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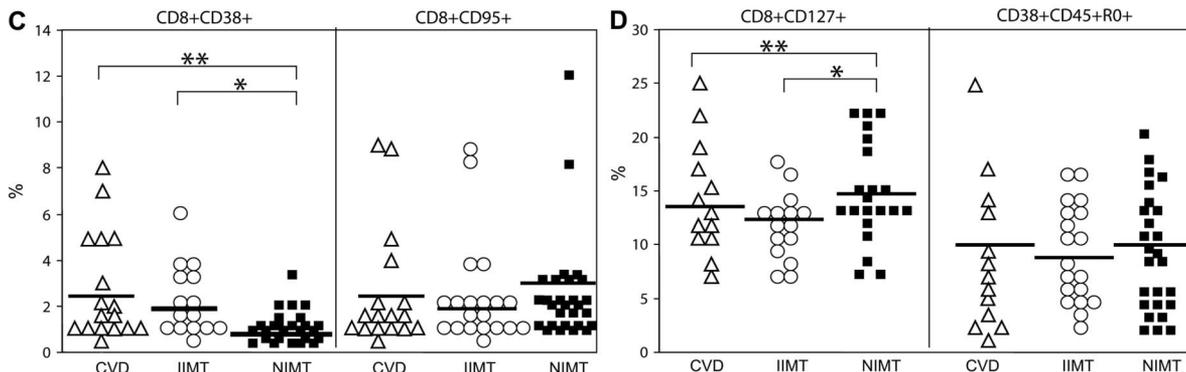
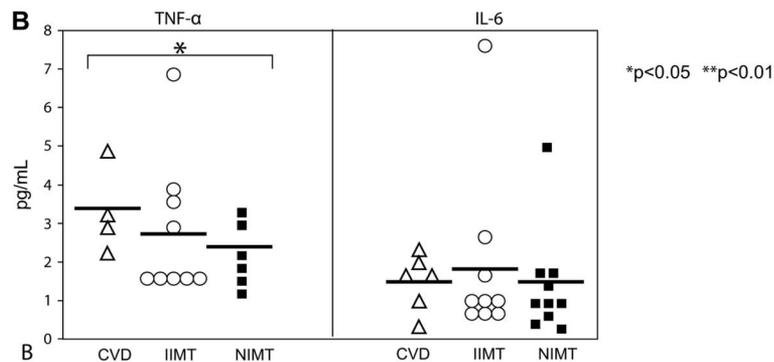
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**A**

Characteristic	CVD, (n=17)	IIMT, (n=19)	NIMT, (n=28)
Age, years	54 ± 3	50 ± 2	46 ± 2
Sex, Male	15	18	23
Time from HIV diagnosis, years	7	11	6
Nadir CD4/μL	202 ± 40	223 ± 54	240 ± 34
Zenith HIV RNA, log cp/mL	5.4 ± 1.7	5.5 ± 4.6	5.7 ± 5.3
Previous AIDS diagnosis			
Yes (%)	4 (24)	8 (42) <sup>c</sup>	3 (11)
Current CD4/μL	447 ± 64	566 ± 87	448 ± 46
Current HIV RNA, log cp/mL	4.2 ± 4	4.5 ± 4	3.5 ± 3
Type of HAART			
2 NRTI+1 PI	4 <sup>a</sup>	11	9
2 NRTI+1 NNRTI	4	7	14
3 NRTI	2	0	1
HAART length, months	44 ± 11 <sup>a</sup>	99 ± 16	67 ± 12
HCV infection			
Yes (%)	1 (6%)	2 (10%)	4 (14%)
Carotid IMT, mm			
Left	NA	1.22 ± 0.06 <sup>c</sup>	0.82 ± 0.03
Right		1.26 ± 0.05 <sup>c</sup>	0.84 ± 0.03
Total cholesterol, mg/dL	212 ± 16	207 ± 11	197 ± 7
HDL cholesterol, mg/dL	46 ± 4	44 ± 2	48 ± 3
LDL cholesterol, mg/dL	134 ± 19	111 ± 9	118 ± 6
TGL, mg/dL	251 ± 66	199 ± 16	219 ± 29
Serum glucose, mg/dL	120 ± 17	97 ± 3	94 ± 3
Arterial Blood Pressure, mmHg ≥ 140/90 (n)	4	2	5
Waist circumference, cm	99 ± 3.5 <sup>b</sup>	94 ± 2 <sup>c</sup>	88 ± 2
Smokers			
Yes (%)	5 (29)	8 (42)	8 (28)
Use of lipid lowering agents			
Yes (%)	4 (23)	4 (21)	5 (18)
Use of glucose lowering agents			
Yes (%)	4 (23) <sup>b</sup>	1 (0.5)	0 (0)
Use of ABP lowering agents			
Yes (%)	7 (41)	4 (21)	5 (18)



**FIGURE 1.** A, Patients' characteristics. HAART, highly active antiretroviral therapy. Patients on HAART at time of study: 10 CVD, 18 IIMT, and 24 NIMT. Fasting serum glucose was measured. Smokers defined as ≥10 cigarettes per death. ABP drugs included calcium antagonists, beta blockers, ACE inhibitors, spartanics, and diuretics; lipid lowering therapy included statins and/or fibrates; glucose lowering therapy included oral glucose lowering agents and/or insulin. <sup>a</sup>P < 0.05 for IIMT vs CVD; <sup>b</sup>P < 0.05 for CVD vs NIMT; <sup>c</sup>P < 0.05 for IIMT vs NIMT. B, Plasma TNF-α and IL-6. C) CD38<sup>+</sup> CD8<sup>+</sup> and CD95<sup>+</sup> CD8<sup>+</sup> percent. D) CD127<sup>+</sup> CD8<sup>+</sup> and CD38<sup>+</sup> 45R0<sup>+</sup> CD8<sup>+</sup> percent. ABP, arterial blood pressure; ACE, angiotensin-converting enzyme; HCV, hepatitis C virus; LDL, low-density lipoproteins; NA, not applicable; TGL, triglycerides. Patients' age and HAART at time of event/ultrasonography.

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## Screening and Treating Cervical Cancer in HIV-Positive Women in Cambodia

### To the Editor:

HIV-positive women have a higher risk of cervical intraepithelial neoplasia (CIN) and cervical cancer than the general population.<sup>1,2</sup> Moreover, the safety and efficacy of human papillomavirus vaccines in HIV-positive women still need to be assessed. Thus, many recommend that cervical precancer lesions be aggressively sought and treated among HIV-positive women.<sup>3,4</sup> Cervical cancer screening is not routinely available in Cambodia, and certainly not for HIV-positive women, despite the fact that

cervical cancer is the most common malignancy in women in the country.<sup>5</sup> In response, Médecins Sans Frontières (MSF) and the Reproductive Health Association of Cambodia initiated a screening program targeting HIV-positive women enrolled in 2 HIV clinics located at 2 provincial referral hospitals. We expected that a screening program targeting HIV-positive women would be effective given that women return regularly for HIV care and benefit from a recall system already in place.

A patient referral system was set up in early 2007 from the HIV clinics supported by MSF to the Reproductive Health Association of Cambodia clinics located nearby. At that time, 2031 women were enrolled in these 2 clinics, of which 1418 were on antiretroviral therapy. Patients were offered screening and treatment free of charge with additional support by MSF, including transportation, accommodation, and food allowance as required.

The choice of the screening strategy and the development of the treatment protocol were guided by the limited availability of diagnostic and therapeutic techniques in the country. Papanicolaou (Pap) test was used as the primary screening method because visual inspection method with acetic acid (VIA) had only been recently introduced in the country and was not well enough developed for a screening program at that time. Biopsy was recommended for women with abnormal cytology and/or with macroscopic lesions. Cytology and biopsy required pathology laboratory services only available at the country's capital, Phnom Penh. As for treatment, the options for the program included cryotherapy, available only in the capital, and hysterectomy, available in the provincial hospitals. Large loop excision of the transformation zone and cold cone biopsy procedures were not readily available in Cambodia at the time the screening was carried out.

The first 200 women screened represented 10% of all HIV-positive women enrolled in the 2 HIV clinics. The median age was 35 years (interquartile range: 30–39 years) and 150 of 200 (75%) were on antiretroviral therapy at the time of the screening. Fifty-three women (25%) had abnormal cytology and 3 macroscopic lesions. Overall,

30 women were diagnosed with CIN grade 1 to 3 and 4 with cervical cancer by biopsy. Fourteen women (26%) were lost to follow up during the screening process and biopsy uptake was 75% (42 of 56). These results were not optimal especially considering the extensive support by MSF provided for referrals including transport, accommodation, and food. Despite this support, women who required biopsy still had to make a 2-day trip to the capital, placing a burden on their families.

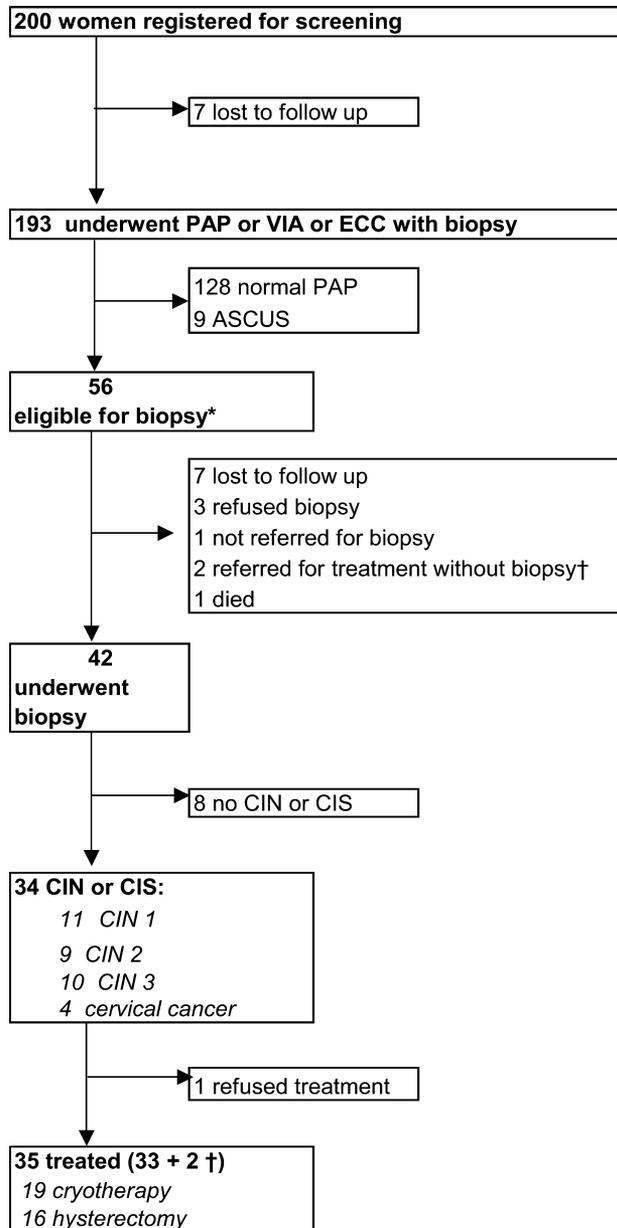
On the other hand, the uptake of treatment (cryotherapy or hysterectomy) after positive biopsy was high at 97% (33 of 34). The availability of hysterectomy at the clinic sites may have contributed to this. Details of the outcomes of women enrolled in the screening and treatment program are shown in Figure 1.

Implementation of a cytology-based (Pap) screening strategy in resource-limited settings, even for a population routinely followed up in a clinic as our cohort, is problematic. It requires trained laboratory pathologists, often only available at the capital level, a system for laboratory quality assurance, and a reliable timely transport mechanism. In our setting, the screening process involved several consultations: a minimum of 2, if Pap test was negative, up to 4 visits if abnormal cytology was detected. This represented a burden for both the women screened and the program. Another challenge posed by the cytology-based screening was the delay in the transport of specimens and reporting the results (an average of 3 weeks in our setting).

In addition, we found that organizing a referral system from the HIV clinic to the reproductive health clinic and tracing and recalling women who needed referral to the capital for biopsy or treatment was time consuming and resource demanding for the program. Screening for cervical cancer in a resource-constrained setting using the conventional Pap test seems just as difficult for HIV-positive women, already enrolled in care, as for women of the general population.<sup>6</sup>

Recently, there have been major advancements in the field of cervical cancer prevention for women in developing countries. The single-visit approach relying on VIA and cryotherapy has been shown to be safe, feasible,

Part of the data were presented at the XVII International AIDS Conference in Mexico on July 8, 2008 and published in the conference abstract book (reference THPDB205).



\* eligible for biopsy: abnormal cytology or macroscopic lesion

† patients treated without biopsy

**FIGURE 1.** Outcome of cervical cancer screening among HIV-positive women. ASCUS, atypical squamous cells of uncertain significance; CIS, carcinoma in situ; ECC, endocervical curettage.

acceptable, and effective when used for women of the general population in resource-limited settings<sup>7,8</sup> but evidence for its value for HIV-positive women is still lacking.

Given the significant increase in the life expectancy of HIV-positive women under highly active antiretroviral therapy, and the unclear impact of highly

active antiretroviral therapy on the incidence and evolution of CIN to date, HIV-positive women deserve more consideration when developing screening and treatment protocols and setting research priorities.

Research is now needed to confirm the value of a single-visit approach, using VIA and cryotherapy, for HIV-

positive women. The risk of overdiagnosis and consequently overtreatment by using VIA as the main screening test is still unclear. Moreover, the efficacy and safety of cryotherapy need further assessment among HIV-positive women. In resource-limited settings, further operational research is also needed on models of integration of cervical cancer prevention and other reproductive health services within HIV programs.

In summary, we have found that the Pap test-based strategy for cervical cancer screening for HIV-positive women in 2 provincial hospital clinics in Cambodia results in a high yield but is resource demanding and challenging to implement even when targeting regular HIV-clinic attendees. We suggest further investigation of the single-visit approach, using VIA and cryotherapy, thereby bypassing the need for cytology-based screening and minimizing referrals and repeated consultations, for HIV-positive women in resource-constrained countries.

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## Can Oral Fluid Testing Be Used to Replace Blood-Based HIV Rapid Testing to Improve Access to Diagnosis in South Africa?

### To the Editor:

In response to a recent letter to the editor of JAIDS<sup>1</sup> that described the high diagnostic accuracy of 2 oral fluid (OF)–based tests for HIV surveillance, in a predominantly HIV subtype C, resource poor environment in southern Africa, we investigated a second study in Johannesburg, South Africa. Much effort is being invested in increasing the access to diagnosis of HIV infection in South Africa. Global access to testing has expanded dramatically since the advent of whole blood–based rapid HIV testing strategies as part of voluntary counseling and testing programs.<sup>2,3</sup> This is particularly relevant in resource-constrained countries where laboratory infrastructure is often poor, skilled phlebotomists and technicians are unavailable, and courier networks poorly developed.<sup>4</sup> The possibility that saliva could be used for HIV screening and diagnosis has been known since 1986,<sup>5</sup> with the best results obtained from OF rich in immunoglobulin G that mostly comes from crevicular

space between the gums and teeth and not from salivary glands.<sup>6–8</sup> Initial poor sensitivity results were also attributed to microorganisms generating proteases that degrade immunoglobulins rapidly or high-viscosity mucins with high viscosity compromising the pipetting process.<sup>8</sup> Improved performance has been described with the collection of OF or oral mucosal transudate (OMT)<sup>9–11</sup> with OF comparable to serum testing.<sup>10–12</sup> OF HIV testing would significantly improve access to testing in the South African voluntary counseling and testing program, and the acceptability of OF collection has been shown in several large community-based surveillance studies in the region.<sup>13</sup> The Namibian study<sup>1</sup> showed the 100% sensitivity and 100% specificity of the OraQuick Rapid HIV-1/2 test (OraSure Technologies, Bethlehem, PA) to be suitable for areas with limited laboratory resources. The 97.1% sensitivity and 99.5% specificity of the OraSure test (OraSure Technologies) used in combination with the Vironostika HIV Uni-Form II plus O enzyme-linked immunosorbent assay (ELISA), (bioMérieux, Durham, NC) was found suitable for complete confidentiality for high-volume batch HIV testing for surveillance. Based on the good sensitivity and specificity of the OraQuick reported in Namibia,<sup>1</sup> and renewed interest expressed by clinicians in South Africa to use alternative samples to blood such as OF for HIV testing (W. D. F. Venter, MD, personal communication 2008), this second investigation was performed in Johannesburg, South Africa (predominantly HIV-1 subtype C). In a cohort of 150 randomly selected patients, we investigated whether OF testing using the OraQuick can be used to replace blood-based HIV rapid testing to improve access to HIV diagnosis in South Africa.

An additional 5 mL K<sub>3</sub> EDTA tube of blood was collected from consenting patients referred for routine phlebotomy tests from the Johannesburg Hospital outpatient department attached to the University of the Witwatersrand HIV antiretroviral (ARV) and surrounding clinics (University of the Witwatersrand Human Ethics Committee approval number M03-05-78). This blood specimen was used for the rapid OraQuick blood

test (according to the manufacturer's instructions within 4 hours post venesection), which for confidentiality reasons was performed in the laboratory adjacent to the clinic. Before leaving the clinic, the patients were asked to swab their upper and lower buccal gums using the OraQuick collection device to collect OMT. The lateral-flow immunoassay result was read by the study nurse 20 minutes after the patient had left the clinic according to the manufacturer's instructions. Confirmatory testing was performed using the automated HIV ELISA 4th generation Abbott AxSYM HIV Ab/Ag assay (Abbott AxSYM; Abbott Laboratories, Wiesbaden-Dellxheim, Germany) according to the manufacturer's instructions, which was considered the gold standard assay for study purposes. This assay simultaneously detects p24 antigen and anti-HIV-1/2 antibody. The result is calculated as an optical density (OD) rate (using negative controls to produce a cutoff value on which the OD of the sample is compared to determine the antibody status). Samples with OD/cutoff values greater than 1.0 are considered as antibody reactive (positive). Samples with nonreactive qualitative results but with a "low positive" AxSYM OD rate reading near the cutoff point were confirmed negative using the manual BioRad Genetic Systems rLAV HIV-1 ELISA (BioRad Laboratories, Redmond, WA). Specimens with an absorbance value of less than the cutoff value are regarded as nonreactive for HIV-1 antibodies. In addition, these samples were also subjected to a nucleic acid RNA polymerase chain reaction viral load test (COBAS AmpliPrep/COBAS Amplicor HIV-1 Monitor; Roche Diagnostics, Branchburg, NJ) to exclude the presence of acute HIV infection. This assay has a dynamic range of 400–750,000 copies per milliliter. Patients were given the option of being contacted and counseled once the reference HIV ELISA results were made available.

Using the Abbott AxSYM 4th generation HIV ELISA assay results, 81 (54%) of the samples were HIV positive and 69 (46%) were classified as HIV negative. Four ELISA results close to the OD cutoff on the AxSYM ELISA were confirmed negative using the BioRad ELISA and showed below

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detectable HIV viral load values by RNA polymerase chain reaction. There was 100% concordance between results obtained with the blood OraQuick test and the AxSYM HIV ELISA test. The OMT OraQuick, however, performed less well. No false-positives were recorded using the OMT OraQuick test, but 3 samples confirmed HIV-positive ELISA samples were recorded as negative using the OMT OraQuick. These results had OD rate values >35 and were all clearly within the positive range of both the ELISA and the OraQuick blood-based assays. The specificity of the OraQuick for OMT was determined as 100% with a sensitivity of 96% (Table 1). The latter was therefore found to be less than the 100% reported in the Namibian study.<sup>1</sup> This therefore presents some concerns over the sensitivity of the OMT assay for routine HIV diagnosis in the South African context. The 3 patients incorrectly identified as HIV negative using the OMT OraQuick were recorded as receiving ARV treatment (3TC, d4T and Efavirenz) with CD4 counts ranging from 287–479 cells per microliter and <Log<sub>2</sub> and Log<sub>5</sub> viral load copies per milliliter.

False-negatives have previously been described using the OraQuick (OMT and sera) device, on patients characterized by early initiation of effective ARV therapy (sensitivity 96%) demonstrating waning serum anti-gp41 titers and Western blot band intensities,<sup>14</sup> or insufficient oral specimen volume collected<sup>1</sup> or the presence of low HIV antibody levels during the early phase of infection. In contrast to other reported studies,<sup>1,15,16</sup> no false-positives were reported in this study using the OraQuick on either OMT or blood samples. OF collection offers the advantages of convenience, economy, and safety and are more acceptable to subjects (including paediatrics<sup>17</sup>) than blood

tests.<sup>6,18–22</sup> Several rapid HIV assays are now Food and Drug Administration approved,<sup>23</sup> but despite poorer sensitivities, the OraSure OF collection device in combination with the Vironostika HIV Uni-Form II plus O ELISA (bioMerieux) has been used widely for population-based surveillance in Southern Africa<sup>24</sup> including a national household survey.<sup>25</sup> Postmarketing surveillance of the OraQuick on whole blood and OF in a large US study (n = 135,724 blood, n = 26,066 OF) showed in accordance with the manufacturer's claim for specificity (100% whole blood and 99.8% OF)<sup>26</sup> that the OraQuick on OF was lower.<sup>15</sup> A second study in the United States also showed slightly more false-positive and false-negative results occurred with OF than whole blood in diverse settings (clinics, labor/delivery wards, and outreach venues), but still considered the Clinical Laboratory Improvement Amendment-waived OF test to provide HIV testing that is acceptable and useful in outreach settings for persons with high risk of infection.<sup>16</sup>

The sensitivity and specificity data from this Johannesburg study confirms that the OMT OraQuick may be suitable for large-scale prevalence studies, particularly community-based surveys in South Africa, and could be used as an alternative to the OraSure collection device in combination with Vironostika ELISA that has previously been used in bulk of these kinds of studies. The use of the OMT OraQuick (with a sensitivity of 96%) for diagnostic purposes in our experience, however, is below that quoted for commonly used blood-based rapid tests.<sup>27,28</sup> For this reason, this assay and other newly introduced OF assays such as the Calypte AWARE HIV-1/2 OMT (Calypte Biomedical, Portland, OR)<sup>29</sup> should not be implemented for diagnostic purposes in our local setting without further evaluation on HIV subtype C.

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**TABLE 1.** Sensitivity and Specificity of the OraQuick OMT and Blood Tests According to the Confirmatory AxSYM ELISA Test

	OraQuick OMT	OraQuick Blood	AxSYM ELISA
Positive tests	78	81	81
Negative tests	72	69	69
Sensitivity	0.96 (96%) CI: 0.928 to 0.991	1.00 (100%)	N/A
Specificity	1.00 (100%)	1.00 (100%)	N/A

N/A, not applicable.

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## Oral Fluid HIV Rapid Testing in Southern Africa Is Suitable for Population-Level Surveillance But May Not Be So for Individual Diagnosis (Invited Response to Scott et al: Can Oral Fluid Testing Be Used to Replace Blood-Based HIV Rapid Testing to Improve Access to Diagnosis in South Africa?)

### To the Editor:

In a Letter to the Editor, Scott et al<sup>1</sup> reported the results of a study that evaluated the diagnostic accuracy of the oral fluid (OF)-based OraQuick (OraSure Technologies, Inc, Bethlehem, PA) rapid test for the detection of HIV antibodies compared with a blood-based reference standard in a cohort of 150 randomly selected outpatients in Johannesburg Hospital, South Africa (with HIV

prevalence of 54%). The study was conducted as a second investigation after our recent evaluation study of OF OraQuick in a population of 273 pregnant women attending antenatal clinics in Namibia (with HIV prevalence of 25.6%), which demonstrated a sensitivity of 100% (95% confidence interval (CI): 94.9 to 100%) and a specificity of 100% (95% CI: 98.2 to 100%), yielding positive predictive value (PPV) and negative predictive value (NPV) of 100%.<sup>2</sup> Based on available data from developing countries,<sup>2–4</sup> we then concluded that the OF OraQuick test is suitable for population-based surveillance in sub-Saharan Africa. Its convenience and user-friendliness make it an attractive option for enhancing the collection of reliable prevalence data among various at-risk populations,<sup>5</sup> although we also recommended ongoing quality assessment of OF HIV testing and the development of safeguards against inadequate OF sampling as well as evaluation of additional applications (such as individual diagnostic testing).<sup>2</sup>

These encouraging findings resulted in a renewed interest from clinicians in South Africa to use OF as an alternative specimen source to blood for individual HIV diagnostic testing.<sup>1</sup> However, the subsequently conducted evaluation study of OF OraQuick by Scott et al<sup>1</sup> found a reduced test sensitivity (96.3%, 95% CI: 92.8 to 99.1), raising concerns about the OF test performance for individual diagnosis. Reasons for the 3 false-negative results are difficult to pinpoint. Our experience (>17,000 OF OraQuick tests) in domestic household surveys and anonymous workplace surveys in Namibia has shown that the correct interpretation of a faint line on the test device, as a (weakly) positive result, might be challenging for staff with relatively little training and experience with OF OraQuick (I. de Beer, personal communication). Other possible explanations include the collection of insufficient OF specimen volume<sup>6</sup> possibly related to instructing clients suboptimally, association with antiretroviral therapy,<sup>1–7</sup> or low HIV antibody levels in early or late infection.

The predictive values (PPV/NPV) are the most important measure of a diagnostic test because they express the probability that a positive/negative test result correctly reflects the presence/

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absence of the condition being tested for, in this case antibody to HIV. The predictive values vary with the disease prevalence in the population from which the person comes. With increasing prevalence, the probability that a person testing positive is truly infected (ie, PPV) increases and the proportion of specimens that are false-positive decreases. Conversely, with increasing prevalence, the probability that a person testing negative is truly uninfected (ie, the NPV) decreases and the proportion of specimens testing false-negative increases. For the purpose of HIV screening, the ideal test would yield no false-negatives, that is, sensitivity and NPV of 100%. Application of the OF OraQuick test with 96.3% sensitivity<sup>1</sup> in populations with varying HIV prevalence, for instance 54% (Scott et al<sup>1</sup>), 25.6% (Namibian study<sup>2</sup>), 18.1% (national adult prevalence in South Africa<sup>8</sup>), 15.3% (national adult prevalence in Namibia<sup>8</sup>), and 5% (overall adult prevalence in sub-Saharan Africa<sup>8</sup>), would yield NPVs of 95.8%, 98.7%, 99.2%, 99.3%, and 99.8%, respectively.

In conclusion, we agree with Scott et al that the reported reduced test sensitivity of OF OraQuick would preclude its use for individual HIV diagnostic testing among high-prevalence groups as present in southern Africa. Their finding adds to concerns about reduced test specificity reported in New York<sup>9</sup> and Boston.<sup>10</sup> Nonetheless, we argue that the extremely high HIV prevalence (54%) in the study population partially explains the reduced NPV found by Scott et al.<sup>1</sup> We here illustrated that the NPV of OF OraQuick is sufficiently high for the purpose of population-level surveillance in southern Africa, such as in antenatal

screening, domestic household surveys, and anonymous workplace surveys. Reliable HIV prevalence studies among various at-risk populations in sub-Saharan Africa are essential for the development and evaluation of effective HIV control initiatives.<sup>11</sup> The use of OF rapid tests is particularly attractive in areas with limited laboratory resources. When using OF OraQuick, we recommend providing appropriate training to laboratory staff, especially in the interpretation of faint lines. In addition, the development of safeguards for adequate OF sampling and studies into possible effects of HIV-1 subtypes are warranted.

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