The influence of concomitant HIV infection on the serological diagnosis of primary syphilis in southern Africa

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The authors investigated the utility of both a non-treponemal (RPR) test and a treponemal (FTA-ABS) test for the diagnosis of primary syphilis during the emergence of the HIV epidemic in southern Africa. The serological tests were performed on 868 patients with genital ulcerations, seen in five centres. While primary syphilis was diagnosed by multiplex PCR in 163 cases (18.8%), the overall RPR and FTA-ABS seroprevalences were 24.3% and 51.5% respectively. The sensitivities of the RPR and FTA-ABS to detect primary syphilis were 69.3% and 89.6% respectively, while the specificities were 86.1% and 58.5% respectively. The performance characteristics of these tests were influenced negatively by concomitant HIV infection and the presence of other genital ulcer disease pathogens in lesions found to be *Treponema pallidum* PCR positive.

The bi-directional nature of the interaction between conventional sexually transmitted infections (STIs) and HIV has been documented in many studies.[1,2] It has come to be recognised that the presence of STIs can increase the risk of acquisition of HIV infection and also promote its transmission, while HIV infection and associated immunodeficiency can alter the natural history and influence the diagnosis and management of conventional STIs.[3-5] The magnitude of the impact of this interaction is more evident in developing countries where the twin epidemics of HIV infection and conventional STIs coexist and produce a vicious cycle that is less evident in more developed societies. During the 1990s, several studies indicated that the incidence of HIV among patients with STIs was significantly higher than those without. This association was more pronounced among those patients presenting with genital ulcer disease (GUD).[6-9]

As a cause of GUD, syphilis is associated with an increased risk of both acquisition and transmission of HIV. Establishment of a definitive diagnosis in cases of primary syphilis is important in order to provide appropriate therapy as soon as possible to prevent spread of both syphilis and HIV (in the co-infected) and reduce the risk of acquisition of HIV (in those not already infected). Unfortunately, techniques to detect *Treponema pallidum* in primary lesions (either by darkfield microscopy, direct immunofluorescence or amplified molecular technologies) are usually not available in most settings in developing countries, and clinicians usually have to rely on serological tests to establish a diagnosis.

Several studies have suggested that concomitant HIV infection may change the performance characteristics of serological tests for syphilis.[10-14] In these studies, we have investigated the utility of both a non-treponemal (RPR) test and a treponemal (FTA-ABS) test for the diagnosis of primary syphilis during the emergence of the HIV epidemic in southern Africa.

Patients and methods

Patients

Eight hundred and sixty-eight patients with genital ulcerations were enrolled in five aetiological studies conducted in Maseru (Lesotho), Johannesburg, Cape Town, Durban and Carletonville STD clinics during the period 1994 - 1999. All patients were of black African ethnicity and the majority (97%) were male. Patients were eligible to enter these studies if they presented with visible genital ulceration(s) of at least 2 mm in diameter with or without inguinal and/or femoral lymphadenopathy. However, they were subsequently excluded if they had a history of treatment with antibiotics, or had received antiviral therapy during the past 7 days, or failed to give oral informed consent for completion of a questionnaire and collection of clinical specimens.

Laboratory methods

In each case, material from the bases of target lesions was collected using a cotton-tipped swab (Medical Wire and Equipment, Corsham, UK). Each swab was then expressed into 0.2 ml sterile distilled water and stored frozen at –70°C until analysed. Subsequently, a multiplex polymerase chain reaction (M-PCR) assay (Roche Molecular Systems) was used to detect specific target sequences of DNA from *T. pallidum*, *Haemophilus ducreyi*, and HSV from the swabs. An aliquot of the processed specimen was also used in a *Chlamydia trachomatis* PCR test (Amplicor, Roche Molecular Systems). All M-PCR assays were performed at the Centers for Disease Control and Prevention, in Atlanta, Georgia, USA, using methods described previously.[15]

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In addition, vacuum collection tubes without anticoagulant were used to collect 10 ml of venous blood from each patient. After collection, blood specimens were allowed to clot at room temperature, centrifuged at 1200 x g for at least 5 minutes and the serum subsequently stored at 4°C or frozen at -70°C. Serological testing for syphilis was performed using both a quantitative non-treponemal RPR test (Immunotrep, Omega Diagnostics, Alloa, Scotland, UK) and a treponemal test (FTA-ABS, Marburg, Germany). HIV testing was performed using a rapid test (Capillus HIV-1/HIV-2, Cambridge Biotech, USA) and all positive samples were confirmed by an ELISA test (AxSYM HIV1/2, Abbott Diagnostics). All indeterminate or low-titre positive specimens were further tested using the Western Blot method (HIV 2.2 Blot, Genelab Diagnostics).

All HIV-seropositive patients were provided with post-test counselling and referred to local HIV clinics for follow-up and appropriate management.

All study protocols were approved by the Committee for Research on Human Subjects, of the University of the Witwatersrand, Johannesburg.

Results

The results of molecular testing to determine the aetiology of the ulcerations seen in the five centres are shown in Table I. Of 868 patients included in these studies, *H. ducreyi* (HD) was detected by M-PCR in 436 (50.2%), *T. pallidum* (TP) in 163 (18.8%), and herpes simplex virus (HSV) in 268 (30.9%), while *C. trachomatis* (CT) was detected by PCR in 29 cases (3.3%). No organism could be demonstrated in 88 cases (10.1%). A single aetiological agent was detected in 672 cases (77.4%), while multiple aetiologies were detected in 108 (12.4%). Among those with a single aetiology, HD was detected in 346 (51%), HSV in 203 (30.2%), TP in 106 (15.8%) and CT in 17 (2.5%) cases. The most common mixed infections involved syphilis, chancroid and genital herpes. Of 163 TP-positive patients, 57 (34.9%) were co-infected with at least one other aetiological agent, namely: 33 (57.9%) with HD, 13 (22.8%) with HSV, and 7 (12.3%) with both. Co-infection with HD and HSV was also common.

Overall, 211 (24.3%) of sera obtained from these patients were reactive in both the RPR and FTA-ABS tests, a further 236 (27.2%) were reactive in the FTA-ABS test alone, and 421 (48.5%) were negative for both. No false-positive RPR reactions were detected. Among RPR-seropositive patients, 137 (64.9%) had a titre of 1:8 or higher (see Fig. 1). The overall geometric mean RPR titre was 1:11.5.

The rates of positive syphilis and HIV serology among patients with different GUD aetiologies are shown in Table II. The highest rate of RPR-seropositivity (69.3%) was found among patients with a positive M-PCR-TP. Of 163 M-PCR-TP-positive patients, the RPR positivity rate was higher in patients with single TP infections (79.2%) than in those with mixed aetiology involving TP and other agents (50.9%) (χ^2 = 14.03, p<0.0001).

The RPR seropositivity rate recorded among patients with a positive M-PCR-TP was significantly higher than that recorded among patients with a negative M-PCR-TP (69.3% v. 13.9% χ^2 = 216.9, p<0.001). The geometric mean RPR titre of sera collected from cases that were solely M-PCR-TP positive was similar to that recorded among cases where ulcerations were M-PCR-TP positive but which had another pathogen detected in the same lesion (1:17.1 v. 1:16.8). However, the geometric mean titre was significantly lower in RPR-seropositive patients with non-treponemal ulcerations (1:5.9) (ANOVA, F statistics = 2.9, p<0.05).

Among all patients included in these studies, the rate of FTA-ABS seropositivity was 51.5%. Of the 657 RPR-seronegative patients, 236 (35.9%) were positive by the FTA-ABS test. As with the RPR test, patients whose ulcerations were only M-PCR-TP positive had a higher FTA-ABS seropositivity rate than those with other aetiologies recorded (96.2% v. 41.5%, χ^2=21.4, p<0.001).

The overall HIV seroprevalence among GUD cases was 46.7%. Of 163 patients who were M-PCR-TP positive, 47 (28.8%) were co-infected with HIV. Among patients with a single aetiology, the lowest HIV prevalence was found among M-PCR-

Table I. Aetiology of GUD among patients in South Africa and Lesotho (1994 - 1999)

Aetiology	No. of patients	%
Single aetiology (*N*=672)		
Primary syphilis	106	12.2
Chancroid	346	39.9
Genital herpes	203	23.4
LGV	17	2.0
Multiple aetiology (*N*=108)		
Primary syphilis & chancroid	33	3.8
Primary syphilis & g. herpes	13	1.5
Primary syphilis, chancroid & g. herpes	7	0.8
Primary syphilis & LGV	3	0.3
Primary syphilis, chancroid & LGV	1	0.1
Chancroid & g. herpes	43	5.0
Chancroid & LGV	6	0.7
G. herpes & LGV	2	0.2
Indeterminate	88	10.1
Total	868	100.0

LGV = lymphogranuloma venereum.

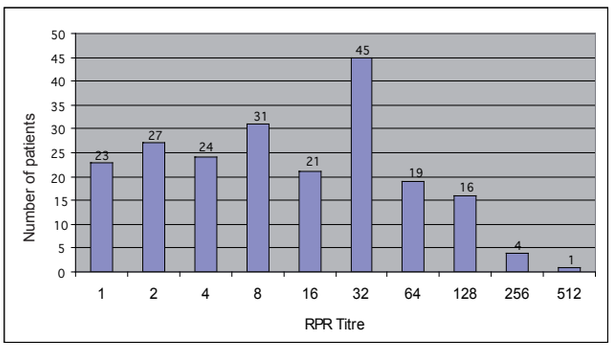

Fig. 1. Distribution of RPR titres in 211 GUD patients with a positive RPR test.

Table II. Syphilis and HIV seroprevalence by aetiology of genital ulcer disease

Aetiology	Number of patients	Both RPR & FTA-ABS +ve		FTA-ABS +ve only		Both RPR & FTA-ABS –ve		HIV-positive	
		No.	%	No.	%	No.	%	No.	%
Single aetiology									
Primary syphilis	106	84	79.2	18	17.0	4	3.8	22	20.8
Chancroid	346	45	13.0	115	33.2	186	53.8	163	47.1
Genital herpes	203	14	6.9	49	24.1	140	69.0	126	62.1
LGV	17	3	17.6	5	29.4	9	52.9	10	58.8
Multiple aetiology									
Primary syphilis & chancroid	33	17	51.5	6	18.2	10	30.3	14	42.4
Primary syphilis & g. herpes	13	6	46.2	6	46.2	1	7.7	7	53.8
Primary syphilis, chancroid & g. herpes	7	4	57.1	2	28.6	1	14.3	3	42.9
Primary syphilis & LGV	3	2	66.7	-		1	33.3	1	33.3
Primary syphilis, chancroid & LGV	1	-		1	100.0		-	-	
Chancroid & g. herpes	43	9	20.9	10	23.3	24	55.8	27	62.8
Chancroid & LGV	6	2	33.3	3	50.0	1	16.7	2	33.3
G. herpes & LGV	2	-		1	50.0	1	50.0	1	50.0
Indeterminate	88	25	28.4	20	22.7	43	48.9	29	33.0
Total	868	211	24.3	236	27.2	421	48.5	405	46.7

TP positive cases (20.8%). However, a higher HIV prevalence rate (43.9%) was recorded in cases that were M-PCR-TP positive but mixed with other aetiological agents (43.9% v. 20.8%, χ^2=9.64, p<0.001). The highest HIV prevalence of 329/617 (53.3%) was found in patients infected with non-treponemal causes of ulceration.

When comparing RPR seropositivity rates by HIV serostatus, the RPR test was positive in 27 HIV-positive/M-PCR-TP-positive cases (57.4%) compared with 86 patients (74.1%) in the HIV-negative/M-PCR-TP group (OR 0.47, 95% CI 0.23 - 0.96, p<0.05). Irrespective of their RPR status, no significant difference in FTA-ABS seropositivity rates was found among HIV-seropositive and -seronegative M-PCR-TP positive patients (83% v. 92.2%, NS). Eight HIV-positive patients (17%) and 9 HIV-negative patients (7.8%) who were M-PCR-TP positive were negative by both serological tests for syphilis. There was no significant difference in the geometric mean titres of the RPR test when comparing HIV-seropositive and -seronegative patients with a positive RPR test (1:13.7 v. 1:18.2, respectively).

Among 163 M-PCR (TP)-positive patients, 106 (65%) were solely infected by TP and 57 (34.9%) had mixed infections with other aetiological agents. Of the 106 patients with a single aetiology, 22 (20.8%) were co-infected with HIV. There was no significant difference in RPR positivity rates between HIV-seropositive and -seronegative groups with a single TP infection (81.8% v. 78.6%, Fisher's exact test NS). However, a significant difference in the RPR seropositivity rates between HIV-positive and -negative groups with mixed GUD aetiologies was detected (36.0% v. 62.5%, χ^2 = 3.9, p<0.05).

Of 705 patients with a negative M-PCR-TP test, 358 (50.8%) were co-infected with HIV, and the overall RPR seropositivity rate was 13.9%. The RPR seropositivity rate in HIV-seropositive patients was 12.3% and in HIV-seronegative patients 15.6% (χ^2=1.6, p=0.2). The RPR seropositivity rate detected in patients with indeterminate laboratory findings was significantly higher than that seen in patients with known aetiologies other than TP (28.4% v. 11.8%, χ^2=17.7, p<0.001), indicating that some cases of primary syphilis were not detected by the M-PCR-TP. This significance was independent of HIV serostatus (HIV-seropositives: 24.1% v. 11.2%, χ^2=4.1, p=0.04; HIV-seronegative group: 30.5% v. 12.5%, χ^2=12.1, p<0.001).

A comparison of the performance of syphilis serology in HIV-seropositive and -seronegative patients with single and mixed infections is shown in Table III. Eighty-eight patients with unknown or indeterminate aetiology were excluded from this analysis. The overall sensitivity of RPR to detect primary syphilis in our setting was 69.3% and its specificity was 86.1%, while the sensitivity and specificity of the FTA-ABS test were 89.6% and 58.5% respectively. Unfortunately, this test, in common with other treponemal tests, exhibited poor specificity for the detection of primary disease because it measures lifetime exposure to *T. pallidum* infection and was found to be reactive in many cases of ulceration that were M-PCR-TP negative. The sensitivity of the RPR test was found to be significantly higher in HIV-seronegative than HIV-seropositive patients (74% v. 57.4%, χ^2= 4.94, p=0.026). The sensitivity of the RPR test was further decreased (to 36%) in HIV-seropositive patients with mixed infections. Among HIV-seropositive patients, there was a significant difference in the sensitivity of the RPR test in patients infected solely by TP when compared to those who had mixed TP infections (81.8% v. 36%, p<0.001). However, this difference was not statistically significant when comparing patients who were HIV-seronegative.

Discussion

As has been demonstrated previously in southern Africa, the utility of both treponemal and non-treponemal serological tests for the diagnosis of primary syphilis is questionable,[16]

Table III. Performance of syphilis serology in the diagnosis of primary syphilis

	RPR			FTA-ABS		
	Single infection	Mixed infection	*p*	Single infection	Mixed infection	*p*
HIV-seropositive						
Sensitivity	18/22 (81.8%)	9/25 (36%)	**0.001**	21/22 (95.5%)	18/25 (72%)	0.08
Specificity	271/299 (90.6%)	21/30 (70%)	**0.002**	175/299 (58.5%)	14/30 (54.5%)	0.58
PPV	18/46 (39.1%)	9/18 (50%)	0.4	21/145 (14.5%)	18/34 (52.9%)	**0.001**
NPV	271/275 (98.5%)	21/37 (56.8%)	**0.001**	175/176 (99.4%)	14/21 (66.7%)	0.27
HIV-seronegative						
Sensitivity	66/84 (78.6%)	20/32 (62.5%)	0.08	81/84 (96.4%)	26/32 (81.3%)	**0.02**
Specificity	233/267 (87.3%)	19/21 (90.5%)	0.9	160/267 (59.9%)	12/21 (57.1%)	0.8
PPV	66/100 (66%)	20/22 (90.9%)	**0.02**	81/188 (43.1%)	26/35 (74.3%)	**0.001**
NPV	233/251 (92.8%)	19/31 (61.3%)	**0.001**	160/163 (98.2%)	6/18 (66.7%)	**0.02**

since many patients with primary syphilis are seronegative (especially by the RPR test), while others with ulcerations caused by other organisms are seropositive (especially by the FTA-ABS test) as a result of previously treated treponemal infections. In these studies, we have examined the effect of HIV co-infection in patients with M-PCR-confirmed primary syphilis on the performance of treponemal and non-treponemal serological tests. Previous studies have reported atypical treponemal and nontreponemal serological responses in HIV-infected individuals with syphilis, such as false negative tests or a delay in seroconversion[12,14] and falsely positive serological responses.[10,11] In the study reported here, no biological false positive RPR reactions were detected among HIV-seropositive patients. This may be a phenomenon which is more associated with intravenous drug abuse than the direct effect of HIV infection; unlike populations studied in more industrialised countries, intravenous drug abuse is rare among patients co-infected with syphilis and HIV in southern Africa.

Only 69.3% of patients with M-PCR-confirmed TP infection were found to be RPR-seropositive, and the rate of RPR seropositivity among primary syphilis patients co-infected with HIV (57.4%) was significantly lower than that recorded among patients without HIV co-infection (74.1%). These results are consistent with a delay in antibody formation occurring as a result of HIV infection and subsequent immunosuppression. However, the rate of RPR seropositivity was also significantly reduced among those patients presenting with TP mixed with other GUD aetiological agents (50.9%) when compared with those with a single TP infection (79.2%). This effect was also seen among HIV-negative patients – with a significantly reduced rate of RPR seropositivity in those with mixed infections. The utility of RPR tests in detecting primary syphilis may be reduced further in areas where both HIV-coinfection as well as GUD with multiple aetiologies are common.

In southern Africa, the HIV epidemic reached maturity in the late 1990s, with an increasing number of immunocompromised patients having been recorded. Most patients with genital herpes who were included in the studies reported here presented with non-vesicular, purulent ulcerations that would usually be diagnosed clinically as chancroid. These chronic ulcers could subsequently serve as a portal of entry for *T. pallidum*, resulting in mixed infections and a delay in development of antibody responses to this 'secondary' infection. Regardless of the underlying mechanism of this decrease in sensitivity, it is clear that caution should be exercised in the interpretation of syphilis serological testing in cases of GUD, particularly in those who are co-infected with HIV, because many cases of primary syphilis may be associated with other causes of GUD.

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