Serological Response to Treatment of Syphilis According to Disease Stage and HIV Status

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(See the Editorial Commentary by Hook, on pages 1623–4.)

Background. Serology is the mainstay for syphilis diagnosis and treatment monitoring. We investigated serological response to treatment of syphilis according to disease stage and HIV status.

Methods. A retrospective cohort study of 264 patients with syphilis was conducted, including 90 primary, 133 secondary, 33 latent, and 8 tertiary syphilis cases. Response to treatment as measured by the Venereal Disease Research Laboratory (VDRL) test and a specific IgM (immunoglobulin M) capture enzyme-linked immunosorbent assay (ELISA; Pathozyme-IgM) was assessed by Cox regression analysis.

Results. Forty-two percent of primary syphilis patients had a negative VDRL test at their diagnosis. Three months after treatment, 85%–100% of primary syphilis patients had reached the VDRL endpoint, compared with 76%–89% of patients with secondary syphilis and 44%–79% with latent syphilis. In the overall multivariate Cox regression analysis, serological response to treatment was not influenced by human immunodeficiency virus (HIV) infection and reinfection. However, within primary syphilis, HIV patients with a CD4 count of <500 cells/µL had a slower treatment response ($P = .012$). Compared with primary syphilis, secondary and latent syphilis showed a slower serological response of VDRL ($P = .092$ and $P < .001$) and Pathozyme-IgM tests ($P < .001$ and $P = .012$).

Conclusions. The VDRL should not be recommended as a screening test owing to lack of sensitivity. The syphilis disease stage significantly influences treatment response whereas HIV coinfection only within primary syphilis has an impact. VDRL test titers should decline at least 4-fold within 3–6 months after therapy for primary or secondary syphilis, and within 12–24 months for latent syphilis. IgM ELISA might be a supplement for diagnosis and treatment monitoring.

With 12 million new cases a year worldwide, syphilis remains a global problem. According to the World Health Organization, >90% of cases occur in developing countries [1]. Rising incidence has been reported in many European countries since the mid-1990s [2].

Similarly in the United States, a resurgence of syphilis has been noted after a nadir in 2000 [3].

For laboratory diagnosis of syphilis and for monitoring treatment response, serological testing is the most important approach. In patients coinfected with human immunodeficiency virus (HIV), atypical serological courses have been reported. Although the clinically defined treatment response does not seem to be influenced by HIV coinfection, serologically defined treatment failure has been reported [4]. This might be a consequence of slower Venereal Disease Laboratory Research (VDRL) test seroreversion in HIV-infected individuals [5]. However, when reviewing previous studies in this field, no firm conclusion can be drawn regarding whether HIV coinfection significantly alters the treatment response. This issue still is important to address, as today most HIV-infected individuals are treated with

highly active antiretroviral therapy and probably have a restored immune system. It is likely that this changed the response to syphilis treatment in HIV patients.

We aimed to compare the serological response to treatment between the different stages of syphilis and to examine the influence of HIV coinfection.

METHODS

Study Population

This study was approved by the ethics committee of Zurich, Switzerland. Retrospectively, we analyzed data from all patients with syphilis at the University Hospital Zurich and City Hospital Triemli who met the following criteria: (1) serological diagnosis of syphilis at the Department of Dermatology between January 1999 and December 2008, (2) start of therapy within 2 weeks after diagnosis, (3) therapy with 1 or 3 doses of benzathine penicillin G (except for patients with tertiary syphilis), and (4) first serological follow-up performed 20–375 days after therapy. Subjects who did not attend follow-up testing within this time period were excluded. We also excluded individuals who did not receive antibiotic treatment of syphilis and patients with incomplete clinical data. Disease stage was classified on the basis of clinical examination and patient history. Most classifications were done at the time when patients visited the clinic, and only a few were classified retrospectively based on the patient chart. Patients were examined to ensure absence or presence of lesions and were considered to have primary syphilis (ulcers at anogenital or oropharyngeal sites and positive serology), secondary syphilis (mucocutaneous skin lesions typical for secondary syphilis and positive serology with or without concomitant ulcers), latent syphilis (no clinical signs of syphilis and positive serology), or tertiary syphilis (combination of clinical and serological findings as well as cerebrospinal fluid analysis). In patients with previous history of syphilis, a ≥4-fold increase of VDRL titer was required to diagnose a new syphilis case. Serological results of the first visit and all follow-up serologies up to 2 years after treatment were collected for 264 patients.

Serological Tests

The VDRL test (Dade Behring, Düdingen, Germany), an immunoglobulin M (IgM) capture enzyme-linked immunosorbent assay (ELISA; Pathozyme Syphilis M Capture, Omega Diagnostic, Alva, United Kingdom), and the *Treponema pallidum* particle agglutination test (TPPA; Fujirebio, Tokyo, Japan) were performed on all sera. The Fluorescent Treponemal Antibody-Absorption test (bioMérieux, Geneva, Switzerland) was performed on 254 of 264 patients. All tests were done according to manufacturers' instructions. If a first-visit serum was VDRL nonreactive, the sample was tested up to a 1:64 dilution to avoid false-negative results due to the prozone phenomenon.

Data Analysis

Therapy start date was defined as baseline. For VDRL analysis, subjects were included if they had a reactive VDRL (titer ≥1:2) at their first visit (baseline) or within 4 weeks after baseline. The endpoint was defined as a 4-fold decrease in the VDRL titer or reversion to nonreactive if the initial titer was 1:2 or 1:4. For Pathozyme-IgM analysis, individuals with a baseline index of ≥0.9 were included. A drop of the Pathozyme-IgM index <0.9 was defined as the endpoint.

Because patients had different follow-up time points, the time of endpoint attainment had to be estimated. Logarithmic curves were calculated for each patient, and the endpoint times were calculated such that y = baseline VDRL/4 or $y = 0$ (for VDRL) or $y < 0.9$ (for IgM). If the calculated logarithmic curve did not fit the observed data ($P > .1$), the actual time to the first follow-up when VDRL was 4-fold decreased (or nonreactive), or when IgM was <0.9, was used instead. Also, if the endpoint was reached prior to the estimated time, the true time was used. If the endpoint was not reached, the patient was defined as censored and the last follow-up was entered as time variable.

For both VDRL and Pathozyme-IgM analyses, we calculated a Cox regression to examine the overall effect on the serological response to treatment of syphilis stage (except tertiary syphilis, as there were too few patients in this group), HIV coinfection according to the CD4 cell count, and reinfection. Sex was excluded as it was not significant in the univariate analysis; therapy was excluded owing to colinearity problems as it is dependent on the syphilis stage. In a second model, we made Cox regression analyses for each syphilis stage (except tertiary syphilis, as there were too few patients in this group) to examine the effect of HIV coinfection and reinfection, within a syphilis stage. All analyses were performed using SPSS software, version 18 (IBM SPSS, Chicago, Illinois).

RESULTS

Clinical and Serological Baseline Characteristics

From January 1999 to December 2008, 456 patients were diagnosed with syphilis. A total of 192 patients were excluded (93 did not attend follow-up serological testing; 42 had incomplete data about therapy or did not receive antibiotic treatment; 20 received nonstandard therapy; 24 started therapy >2 weeks after diagnosis; and 13 had incomplete clinical data). Characteristics of the remaining 264 patients are shown in Table 1 (see also Supplementary Table 1). Of the included patients, 92% were men, 42% were known to be HIV positive, and 13% had a history of previous syphilis (ie, they were considered reinfected). HIV coinfection was significantly associated with male sex ($P = .001$), hepatitis B infection ($P = .031$), history of previous syphilis ($P = .002$), higher number of follow-up visits

Table 1. Characteristics of the Study Population, Overall and According to HIV Status

Characteristic	HIV Positive (n = 112)	HIV Negative (n = 152)	Total (N = 264)	P Value
Male sex	110 (98)	133 (88)	243 (92)	.001[a]
Age, years, median (mean)	38 (38)	37 (39)	38 (38)	NS[b]
Stage of syphilis				.009[c]
Primary	27 (24)	63 (41)	90 (34)	
Secondary	62 (55)	71 (47)	133 (50)	
Tertiary	3 (3)	5 (3)	8 (3)	
Latent	20 (18)	13 (9)	33 (13)	
CD4 cell count, No.				
>500 cells/µL	29	NA	NA	NA
200–500 cells/µL	68	NA	NA	NA
<200 cells/µL	3	NA	NA	NA
Unknown	12	NA	NA	NA
Coinfections				
Hepatitis B virus infection	4 (4)	0	4 (2)	.031[a]
Hepatitis C virus infection	4 (4)	1 (1)	5 (2)	NS[a]
Gonorrhea	1 (1)	3 (2)	4 (2)	NS[a]
Chlamydia	1 (1)	0	1 (0)	NS[a]
History of syphilis	23 (21)	11 (7)	34 (13)	.002[a]
Therapy				<.001[a]
1 dose benzathine penicillin G, 2.4 mU i.m.	15 (13)	100 (66)	115 (44)	
3 doses benzathine penicillin G, 2.4 mU i.m., 1-week intervals	94 (84)	47 (31)	141 (53)	
Aqueous crystalline penicillin G, daily 6 × 3–4 mU i.v. for 10–14 days	3 (3)	5 (3)	8 (3)	
No. of follow-up visits, mean	4.83	3.71	4.19	<.001[d]
Duration of follow-up, mean, days	460	347	395	<.001[b]

All data are No. (%) unless otherwise specified.

Abbreviations: HIV, human immunodeficiency virus; i.m., intramuscular; i.v., intravenous; NS, not significant; NA, not applicable.

[a] Fisher exact test.

[b] t test.

[c] Two-sided column proportions z test with significance level of .05 and Bonferroni correction. Patients with primary syphilis are significantly less often HIV-positive than patients with secondary or latent syphilis.

[d] Exact Mann-Whitney test.

($P < .001$), and longer ($P < .001$) mean duration of follow-up (Table 1). HIV coinfection was also significantly associated with clinical stage of syphilis ($P = .009$); patients with primary syphilis presented less frequently with HIV infection than did patients with secondary or latent syphilis. Concerning therapy, only 7 of 27 HIV patients with primary syphilis received 1 dose of benzathine penicillin G, whereas 20 received 3 doses.

VDRL, Pathozyme-IgM, and TPPA baseline characteristics are shown in Tables 2 and 3. Initial VDRL and TPPA titers were significantly lower ($P < .001$) and more often negative ($P < .001$) in primary syphilis patients compared to those with secondary, tertiary, or latent syphilis. Thirty-eight of 90 (42%), 0 of 133 (0%), 0 of 8 (0%), and 4 of 32 (12%) patients with primary, secondary, tertiary, and latent syphilis, respectively, had a negative VDRL test at their first visit. Six of 90 (7%)

patients with primary syphilis had negative TPPA and VDRL tests; the Pathozyme-IgM tests, however, were positive (median index, 1.42 [range, 1.23–3.00]). Patients with tertiary and latent syphilis showed a significantly lower ($P < .001$) Pathozyme-IgM baseline as compared to primary and secondary stage patients. Four of 90 (4%), 12 of 133 (9%), 3 of 8 (38%), and 7 of 33 (21%) patients with primary, secondary, tertiary, and latent syphilis, respectively, had negative Pathozyme-IgM tests on the first visit. Of the 4 patients with primary-stage symptoms and negative IgM ELISA, 3 had a history of previous syphilis. Across all stages of syphilis, 8 of 34 (24%) reinfected individuals showed a negative IgM test.

Serological Response to Treatment

For VDRL analysis, 214 subjects with an initially positive titer were included. Based on Kaplan-Meier analysis, the median

Table 2. Serological Results at the Time of Diagnosis According to Clinical Stages of Syphilis

Baseline	Primary Syphilis (n = 90)	Secondary Syphilis (n = 133)[a]	Tertiary Syphilis (n = 8)	Latent Syphilis (n = 33)	Total (N = 264)	P Value[b]
VDRL						
Median titer	1:2	1:32	1:48	1:32	1:32	<.001
Interquartile range	1:0–1:16	1:32–1:64	1:16–1:128	1:4–1:64	1:4–1:64	
Negative, No (%)	38 (42)[c]	0	0	4 (12)[d]	42 (16)	<.001
TPPA						
Median titer	1:640	1:20480	1:40960	1: 40960	1:10240	<.001
Interquartile range	1:160–1:3200	1:5120–1:81 920	1:20 480–1:266 240	1:5120–1:143 360	1:1280–1:40 960	
Negative, No. (%)	6 (7)[e]	0	0	0	6 (2)	.015
Pathozyme-IgM						
Median titer	3.34	>3.50	2.00	1.39	>3.50	<.001
Interquartile range	1.78 to >3.50	2.47 to >3.50	<0.9 to >3.50	0.97–3.44	1.71 to >3.50	
Negative, No. (%)	4 (4)[f]	12 (9)[g]	3 (38)[h]	7 (21)[i]	26 (10)	.003

Abbreviations: HIV, human immunodeficiency virus; IgM, immunoglobulin M; TPPA, *Treponema pallidum* particle agglutination test; VDRL, Venereal Disease Research Laboratory test.

[a] In 1 patient, TPPA was not performed.

[b] Kruskal-Wallis test for median titers and Fisher exact test for number of negatives.

[c] Two of these 38 patients were reinfected and 9 were HIV infected (1 of whom had a reactive VDRL test 24 days after baseline). One also had a negative Pathozyme-IgM test.

[d] None of these 4 patients were reinfected and 3 were HIV infected. One also had a negative Pathozyme-IgM test.

[e] All 6 patients had a negative VDRL and a positive Pathozyme-IgM test. Two subjects were HIV infected.

[f] One patient was also negative for VDRL and none for TPPA. Three of these 4 patients were reinfected.

[g] Two of these 12 patients were reinfected and 7 were HIV infected.

[h] One of these 3 patients was reinfected and 2 were HIV infected.

[i] Two of these 7 patients were reinfected and 4 were HIV infected. One also had a negative VDRL test.

Table 3. Serological Results at the Time of Diagnosis According to HIV Status

Baseline	HIV Positive (n = 112)	HIV Negative (n = 152)	Total (N = 264)	P Value[a]
VDRL				
Median titer	1:32	1:16	1:32	.001
Interquartile range	1:8–1:64	1:3–1:64	1:4–1:64	
Negative, No. (%)	12 (11)	30 (20)	42 (16)	NS
TPPA				
Median titer	1:20 480	1: 5120	1:10 240	.002
Interquartile range	1: 2560–1:81 920	1: 640–1:40 960	1: 1280–1:40 960	
Negative, No (%)	2 (2)	4 (3)	6 (2)	NS
Pathozyme-IgM				
Median titer	3.42	>3.50	>3.50	NS
Interquartile range	1.52 to >3.50	1.87 to >3.50	1.71 to >3.50	
Negative, No. (%)	16 (14)	10 (7)	26 (10)	NS

Abbreviations: IgM, immunoglobulin M; NS, not significant; TPPA, *Treponema pallidum* particle agglutination test; VDRL, Venereal Disease Research Laboratory test.

[a] Mann-Whitney test for median titers and Fisher exact test for number of negatives.

time to endpoint (ie, a 4-fold drop of the titer or reversion to nonreactive) was 37 days (95% confidence interval [CI], 29–45 days) for primary, 49 days (95% CI, 46–52 days) for secondary, and 68 days (95% CI, 25–112 days) for latent syphilis. The cumulative serological response to treatment is shown in Table 4. For example, 3 months after treatment, 85%–100% of patients with primary syphilis had reached the endpoint, as compared to 76%–89% with secondary syphilis and 44%–79% with latent syphilis. In the overall multivariate Cox regression analysis, VDRL serological response to treatment was

influenced by syphilis stage but not by HIV infection and reinfection (Table 5). Compared to primary syphilis, latent syphilis showed a significantly slower treatment response (hazard ratio [HR], 0.34 [95% CI, .2–.57]) and secondary syphilis showed a trend to a slower response (HR, 0.74 [95% CI, .53–1.05]). In the second model, when Cox regression analyses were performed for each syphilis stage, HIV-coinfected patients with primary syphilis and a CD4 count of <500 cells/μL showed a significantly slower treatment response compared with HIV-negative patients (HR, 0.37 [95% CI, .17–.81];

Table 4. Serological Response to Treatment of Venereal Disease Research Laboratory and Pathozyme Immunoglobulin M According to Clinical Stage of Syphilis and HIV Status[a]

Baseline	Primary Syphilis	Secondary Syphilis	Latent Syphilis	HIV Uninfected	HIV Infected
VDRL	(n = 52)	(n = 133)	(n = 29)	(n = 117)	(n = 97)
At 90 days	92 (85–100)	83 (76–89)	62 (44–79)	86 (80–92)	77 (68–85)
At 180 days	100	99 (97–100)	76 (60–91)	96 (93–99)	94 (90–99)
At 270 days	100	100	83 (69–97)	98 (96–100)	97 (95–100)
At 360 days	100	100	93 (84–102)	98 (96–100)	100
At 540 days	100	100	93 (84–102)	98 (96–100)	100
Pathozyme-IgM	n = 86	n = 121	n = 26	n = 138	n = 95
At 90 days	35 (24–45)	22 (14–29)	31 (13–49)	26 (19–33)	30 (21–39)
At 180 days	66 (55–76)	44 (35–54)	46 (27–66)	52 (43–61)	54 (43–64)
At 270 days	81 (72–90)	54 (45–64)	68 (48–87)	62 (53–71)	70 (60–80)
At 360 days	87 (79–95)	62 (52–72)	74 (54–94)	73 (64–81)	75 (66–85)
At 540 days	94 (87–101)	79 (69–90)	74 (54–94)	85 (75–95)	86 (77–95)
At 720 days	100	100	74 (54–94)	92 (82–103)	100

Patients with tertiary syphilis were excluded.

Abbreviations: HIV, human immunodeficiency virus; IgM, immunoglobulin M; VDRL, Venereal Disease Research Laboratory test.

[a] Data are estimated percentage of cases (estimated 95% confidence interval) based on Kaplan-Meier analysis.

Table 5. Factors (Cox Regression Results) Determining Serological Response to Treatment for Venereal Disease Research Laboratory[a] and Pathozyme-Immunoglobulin M[b]

Factor	VDRL				Pathozyme-IgM			
	Total (%)	Hazard Ratio	95% CI	P Value	Total (%)	Hazard Ratio	95% CI	P Value
Syphilis stage								
Primary	50 (24.4)	1			82 (36.4)	1		
Secondary	127 (62.0)	0.747	.532–1.048	.092	117 (52.0)	0.531	.373–.757	<.001
Latent	28 (13.7)	0.338	.199–.573	<.001	26 (11.6)	0.469	.260–.846	.012
Reinfection								
No	177 (86.3)	1			200 (88.9)	1		
Yes	28 (13.7)	0.846	.538–1.331	.469	25 (11.1)	1.576	.879–2.826	.127
HIV status (CD4 cell count)								
Negative	117 (57.1)	1			138 (61.3)	1		
Positive (≥500 cells/µL)	25 (12.2)	1.266	.786–2.037	.332	27 (12.0)	1.029	.626–1.693	.910
Positive (<500 cells/µL)	63 (30.7)	0.825	.598–1.138	.241	60 (26.7)	1.373	.935–2.015	.106

Patients with tertiary syphilis were excluded.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; IgM, immunoglobulin M; VDRL, Venereal Disease Research Laboratory test.

[a] For VDRL, n = 205 (51 missing).

[b] For Pathozyme-IgM, n = 225 (31 missing).

$P = .012$). There was no significant influence of HIV coinfection in patients with secondary ($P = .2$) or latent ($P = .41$) syphilis.

A total of 190 patients were followed for at least 1 year or until they had seroreverted for the VDRL test (ie, had a nonreactive VDRL test). Of these, 153 (81%) had seroreverted within a year, 12 (6%) had seroreverted at their first visit after 1 year, and 25 (14%) still had a positive VDRL after 1 year. Of these 25, there were significantly more reinfected patients (28% as opposed to 10%, $P = .022$) and later stages of syphilis (eg, latent or tertiary stage; 52% as opposed to 9%, $P < .001$), when compared with the 153 patients with seroreversion (as analyzed with Fisher 2-sided exact test; data not shown). None of these 25 patients had evidence for insufficient therapy. All but 3 of these patients had initially high titers (≥1:16), which decreased over time. Fourteen patients seroreverted during further follow-up, the last seroreverting 4.2 years after treatment. Compared to this, 11 patients maintained a positive VDRL at the last follow-up, 1.1–3 years after treatment.

For the Pathozyme-IgM test analysis, 233 subjects with an initial positive index were included. The median time to endpoint (ie, a drop below the cutoff index) was 130 days (95% CI, 108–153 days) for primary, 245 days (95% CI, 138–352 days) for secondary, and 202 days (95% CI, 133–271 days) for latent syphilis. One year after treatment, 13%, 38%, and 26% of patients with primary, secondary, and latent syphilis, respectively, did not reach the endpoint (Table 4). In the multivariate analyses, treatment response was not influenced by HIV coinfection ($P = .11$ for those with a CD4 count of <500

cells/µL) or reinfection ($P = .13$), but by clinical stage. Patients with secondary (HR, 0.53 [95% CI, .37–.76]) and latent (HR, 0.47 [95% CI, .26–.85]) syphilis showed a slower treatment response compared to patients with primary syphilis (Table 5). Also, in the second Cox regression analyses performed for each syphilis stage, HIV coinfection had no effect on response time (P value for HIV coinfection was .47, .2, and .27 in primary, secondary, and latent syphilis, respectively).

DISCUSSION

Our study provides evidence that a combination of the TPPA test and an IgM ELISA is superior to the VDRL test for diagnosis of syphilis. Furthermore, the syphilis disease stage significantly influences treatment response, whereas HIV coinfection has an impact on the response only in primary syphilis.

Clinical and Serological Characteristics at Time of Diagnosis

We found a high rate of HIV and syphilis coinfection, which is in agreement with other reports [6, 7]. This patient group presented more often with latent or secondary syphilis, and a substantial proportion were men who have sex with men, as earlier described by the Swiss HIV Cohort Study [8].

As expected, we found significantly lower VDRL and TPPA titers in early stages of syphilis than in later stages. Interestingly, 38 of 90 patients presenting with primary syphilis symptoms had a negative VDRL test result. In 37 of these patients, the initially positive treponemal IgM declined after therapy, proving that the VDRL result was false-negative. Thus, VDRL test

sensitivity was only 58% for primary syphilis, which is lower than previously reported sensitivities of between 66% and 87% [9–11]. Despite this, the VDRL test has been used for first-line screening in the United States [9], and is recommended in textbooks [12]. However, in recent years a paradigm shift is ongoing in the United States toward using sensitive and specific enzyme immunoassays (EIAs) and chemiluminescence assays for screening [13]. In Europe, treponemal tests (EIA or TPPA), which have been shown to be more sensitive, are recommended as single screening tests [14].

In our laboratory, an IgM ELISA is performed for all suspected primary syphilis cases in addition to the TPPA, our standard screening test. The sensitivities of the TPPA and the Pathozyme-IgM ELISA in primary syphilis were 93% and 96%, respectively. Six patients with negative TPPA and VDRL yielded positive IgM ELISA results. The sensitivity when combining TPPA with the IgM test was 100%. We therefore suggest that in cases of suspected early infection, specific IgM ELISA should be used in addition to other screening tests. It is important that clinicians communicate the suspicion of an early infection to the laboratory.

Serological Response to Treatment
As most of our HIV-infected patients probably had a restored immune system, we would not expect to find a significant difference in regard to the serological response rate. Indeed, the response rate of the VDRL test was high in both HIV-positive and HIV-negative patients. In multivariate analyses, HIV coinfection did not influence overall time to serological response after treatment. However, when stratified by syphilis stage, within primary syphilis, HIV-coinfected patients with low CD4 cell count showed significantly slower treatment responses than HIV-negative patients. The majority of previous studies, including several prospective [5, 10, 15–17] and retrospective studies [18–21], concluded that serologic failure or time to serologic response of nontreponemal tests (ie, VDRL, rapid plasma reagin) was not associated with HIV infection even if analyses were stratified by syphilis stage [17, 19, 20]. Other studies, however, including one prospective trial [4], found a significant association [4, 22–24] or a trend [25] between HIV status and response to treatment (ie, HIV-positive patients had a higher risk for serofailure or a longer time to serological response). Three of these studies also presented data that were stratified by syphilis stage and demonstrated that, compared with HIV-negative patients, HIV-positive patients had (1) a higher risk for serofailure only if they had primary or early syphilis [4, 22, 24], and (2) a slower response in latent syphilis [22], or, conversely, slower response in primary syphilis [4], which is in line with our results. Overall, there seems to be a trend toward a slower response of nontreponemal tests in HIV-coinfected patients. However, data in support of making a clear distinction between HIV-positive and HIV-negative patients as recommended by the European guidelines for management of syphilis are scarce [14].

We observed faster serological responses to treatment in earlier stages of syphilis. One year after treatment, 7% (95% CI, 0%–16%) of patients with latent syphilis still showed serological failure in the VDRL test. In multivariate analyses, latent syphilis was a significant predictor for a slower response of the VDRL titer. Previous studies also reported slower response rates in later stages of syphilis [17, 19, 22, 23, 26]. On the basis of our observations, we would propose, similar to the Centers for Disease Control and Prevention guidelines [27], that nontreponemal test titers should decline 4-fold within 3–6 months after therapy for primary or secondary syphilis, and within 12–24 months for latent syphilis.

Of interest is our finding that 81% of patients had seroreverted within a year after treatment, that is, the VDRL test became nonreactive, which is higher than the previously reported proportion of 13%–44% [5, 15, 26]. Almost half of the remaining patients never seroreverted (ie, they had a positive VDRL test at the last follow-up). We suggest, as previously reported [5], that patients with persistently low VDRL titers may be considered successfully treated.

The Pathozyme-IgM ELISA also proved reliable for monitoring the treatment response. However, the response rate was markedly slower than that for the VDRL test. Previous studies [28, 29] of another commercial IgM ELISA (Mercia IgM EIA) reported negative test results 12 months after treatment in 92%–100% of patients presenting with early syphilis, compared with 62%–87% in our study. A specific IgM ELISA might be especially valuable for monitoring patients with an initially negative VDRL test (mainly primary syphilis cases) and in patients with a slow decline or persisting low VDRL reactivity.

Our retrospective study has limitations: (1) A selection bias is of concern because 42% of patients were excluded owing to missing information on follow-up, treatment, or clinical data or because they did not receive standard therapy. (2) Because of the retrospective nature of the study, patients had different follow-up time points. (3) A large number of cases of the latent syphilis group involved patients with syphilis of unknown duration (ie, it is not known whether they had early or late latent syphilis). (4) It is difficult to ascertain whether a patient has experienced reinfection or relapse due to treatment failure. However, we assume that at least 77% of these patients had a reinfection as they had documented previous residual antibodies or presented with a genital ulcer. (5) We cannot ascertain that the patients did not use other antibiotics for other reasons between follow-up visits. Finally, although this is one of the largest studies in the field, the small number of events still impairs statistical power.

CONCLUSIONS

For primary syphilis, the VDRL test should not be recommended as first-line screening test because of its lack of sensitivity. In contrast, both the TPPA test and IgM ELISA were sensitive and the combined sensitivity reached 100%. HIV coinfection did not influence the overall time to serological response to treatment in the VDRL test. However, within primary syphilis, HIV patients with a CD4 count of <500 cells/μL had a slower treatment response. The clinical stage of syphilis had a significant impact on the serological response; compared with primary stage syphilis, latent stage syphilis showed a slower treatment response. Generally, the VDRL titer should decline at least 4-fold within 3–6 months after therapy for primary or secondary syphilis, and within 12–24 months after therapy for latent syphilis. Patients with persistently low VDRL titers after treatment may be considered to be successfully treated. The Pathozyme-IgM ELISA proved to be a reliable supplement for monitoring the treatment response.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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