

# Lack of antiretroviral therapy is associated with higher risk of neurosyphilis among HIV-infected patients who remain serofast after therapy for early syphilis

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## Abstract

Resolution of clinical symptoms and at least 4-fold decline in nontreponemal antibody titers after treatment of syphilis is regarded as “proof of cure.” However, a substantial proportion of patients demonstrates <4-fold reduction of titers after recommended therapy (serofast state). It remains unclear whether the serofast state is indicative of persistence of bacteria or only a residual immune response.

The aim of the present study was to determine the association between the serofast state and the risk of neurosyphilis in human immunodeficiency virus (HIV)-infected individuals and to identify potential predictors of neurosyphilis.

Thirty-three patients with early syphilis and HIV co-infection were included. One year after the recommended treatment of syphilis, all patients who did not achieve proper serologic response underwent lumbar puncture and cerebrospinal fluid (CSF) examination.

Twelve months after completing therapy for syphilis, the proportion of patients with serofast state after syphilis therapy was 36.4% (n = 12). Among them, 5 (41.7%) had neurosyphilis. Individuals who remained serofast and had neurosyphilis (n = 5) when compared to those without neurosyphilis (n = 7) were characterized by significantly lower CD4+ cell count and higher HIV RNA levels ( $P < .05$ ). Moreover, a significantly higher proportion of patients with neurosyphilis was not receiving antiretroviral therapy (ART). The nonreceipt of ART independently increased the risk of neurosyphilis in the analyzed group of serofast HIV-infected patients (odds ratio = 4.5; 95% confidence interval 1.5–13.59,  $P = .003$ ).

Patients co-infected with HIV require careful serologic and clinical follow-up after therapy for syphilis. In all of the patients who do not respond serologically after treatment for syphilis, especially in those who are not receiving ART, lumbar puncture and CSF examination should be considered.

**Abbreviations:** ART = antiretroviral therapy, CDC = Centers for Disease Control and Prevention, CNS = central nervous system, CSF = cerebrospinal fluid, HIV = human immunodeficiency virus, RPR = rapid plasma reagin assay, TPHA = *Treponema pallidum* hemagglutination assay, VDRL = Venereal Disease Research Laboratory assay, WBC = white blood cell.

**Keywords:** antiretroviral therapy, human immunodeficiency virus, neurosyphilis, penicillin, serofast

## 1. Introduction

Since 2000, the overall number of cases of syphilis has increased worldwide. Rates are highest among men, and 75% of patients are men who have sex with men; more than a half of these individuals are human immunodeficiency virus (HIV) infected.<sup>[1]</sup>

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Neurosyphilis, which is the involvement of the central nervous system (CNS) by *Treponema pallidum* subsp *pallidum* (an etiologic agent of syphilis), is one of the most feared complications of syphilis. Some recent studies demonstrated that alteration of the immune system during HIV infection appears to enhance the persistence of bacteria in the CNS, which is associated with more frequent occurrence of neurosyphilis in HIV-infected individuals.<sup>[2–4]</sup>

Parental penicillin is the therapy of choice at every stage of syphilis, but assessment of treatment efficacy relies almost entirely on serologic tests. Appropriate serologic response in syphilis has been defined by the Centers for Disease Control and Prevention (CDC) as a 4-fold or greater decline in the titer of a nontreponemal assay such as the rapid plasma reagin assay (RPR), when occurring 6 to 12 months after initiation of treatment.<sup>[1]</sup> However, in approximately 20% of adequately treated patients with early syphilis, the titer in the nontreponemal assay during follow-up testing does not meet the criteria of a proper response and does not decrease considerably, without any clinical evidence of treatment failure or reinfection. This condition is referred to as “serofast state syphilis.”<sup>[5]</sup> To date, the pathogenesis and clinical significance of the serofast state is unknown.

So far, only a limited number of studies have tried to find an association between an improper serologic response after therapy for syphilis and risk of neurosyphilis.<sup>[6–8]</sup> The results are inconclusive, presumably due to variations in enrollment of patients at different stages of syphilis and inconsistent definitions of neurosyphilis and serologic nonresponse. To the best of our knowledge, none of these studies focused on HIV-infected individuals who remain serofast after appropriate treatment for syphilis.

Thus, the aim of this study was to assess the incidence of neurosyphilis in serofast HIV-infected individuals and to determine likely predictors of CNS involvement.

## 2. Methods

### 2.1. Characteristics of the patients and study design

The HIV-infected patients with secondary and early latent syphilis ( $n=33$ ) during the 1st episode of disease were enrolled at the Department of Dermatology of the Jagiellonian University Medical College (Krakow, Poland) between 2016 and 2018. Study subjects were not treated with antibiotics or immunosuppressive agents during the preceding 6 months. Patients with other chronic inflammatory disorders (e.g., autoimmunity) were also excluded. At the time of enrollment, all patients were positive both for nontreponemal and treponemal tests, and disease staging was determined based on clinical history and physical examination according to CDC recommendations.<sup>[11]</sup> A certified neurologist also evaluated all study participants to assess neurologic symptoms. HIV infection was detected at enrollment, as none of the study participants were aware of their HIV status. Antiretroviral therapy (ART) was introduced in 28 of the included individuals (84.8%). The remaining 5 refused to initiate treatment.

All patients were administered a single dose of intramuscular benzathine penicillin (2.4 million units) as per the recommendations. Patients were followed for 12 months, with assessment of serologic (i.e., RPR blood testing) and clinical response every 3 months. Additionally, every 3 months the HIV RNA levels (viral load [VL]) and CD4+ cell count was determined.

Serology data indicated that 12 months after completing the therapy of syphilis, 12 out of 33 individuals did not achieve proper serologic response, defined as  $\geq 4$ -fold decline in RPR titer when compared to the pretreatment values. In all serologically nonresponding patients ( $n=12$ ), neurologic reexamination, lumbar puncture, and cerebrospinal fluid (CSF) sampling were performed. CSF samples were sent for routine examination (i.e., white blood cell [WBC] count, protein, and glucose concentration) and serology testing (i.e., Venereal Disease Research

Laboratory [VDRL], *Treponema pallidum* hemagglutination assay [TPHA]).

Neurosyphilis was defined as reactive CSF-VDRL or elevated CSF-WBC (i.e.,  $\geq 20$  cells/ $\mu$ L) and elevated CSF protein concentration (i.e.,  $\geq 45$  mg/dL) according to the CDC criteria for HIV-infected individuals. The patients ( $n=12$ ) were classified as neurosyphilis group ( $n=5$ ) and no-neurosyphilis group ( $n=7$ ).

The study was approved by the Jagiellonian University Bioethics Committee (approval number KBET/164/B) and written informed consent was obtained from all participants.

### 2.2. Statistical analysis

Statistical analysis was carried out using STATISTICAL 7.1 PL software (TIBCO Software Inc, Palo Alto, CA). If not stated otherwise, data were expressed as median and minimum–maximum values. Continuous variables were compared with the Mann–Whitney  $U$  test. The Chi-squared test or the Fisher exact test was used for the dichotomous variables. To identify independent factors, a multivariable logistic regression analysis was used. A  $P$ -value  $< .05$  was considered statistically significant.

## 3. Results

Thirty-three previously healthy male patients aged 18 to 52 years were enrolled in this study. The patients were in their 1st episode of early syphilis, staged as secondary syphilis (42.4%) or early latent syphilis (57.6%), and concomitant HIV infection was diagnosed. At the time of enrollment, the mean CD4+ count and HIV load were as follows: 579 cells/ $\mu$ L (min–max: 205–1294) and 57,300 copies/ $\mu$ L (min–max: 224–1,370,000), respectively. All individuals were administered intramuscular benzathine penicillin (2.4 million units). Additionally, ART was offered to all patients. However, 5 of them (15.1%) refused this treatment.

Six months after completing syphilis therapy, 18 patients (54.5%) did not achieve at least 4-fold decline in RPR titer when compared to the pretreatment values. This proportion decreased to 36.4% at 12 months after therapy. At this stage, the patients were stratified into serofast state group ( $n=12$ ) and serologic-cured group ( $n=21$ ). Characteristics of both groups are shown in Table 1. A significantly lower proportion of patients who remained serofast 12 months after treatment of syphilis was receiving ART ( $P < .05$ ). Moreover, HIV-infected individuals with proper serologic response after syphilis treatment were characterized as having a significantly higher CD4+ count when compared to those with serofast state ( $P = .01$ ) (Table 1).

Twelve months after completing the syphilis treatment neurologic examination revealed no symptoms in all serofast

**Table 1**

**Characteristics of HIV-infected patients from serofast state group and serologic-cured group.**

	Serofast group ( $n=12$ )	Serologic-cured group ( $n=21$ )	$P$ -value
Age (min–max), y	32 (24–52)	29 (18–38)	NS
Baseline RPR (min–max)	32 (16–512)	64 (16–1048)	NS
Early latent syphilis, $n$ (%)	8 (66.7)	12 (57.1)	NS
Macular exanthem on the trunk, $n$ (%)	4 (33.3)	8 (38.1)	NS
Papular exanthem on the palms and soles, $n$ (%)	2 (16.7)	1 (4.8)	NS
ART; $n$ (%)	8 (66.7)	20 (95.2)	.047
CD4+ count (min–max), cells/ $\mu$ L	551 (188–1313)	812 (438–1341)	.01
VL (min–max), copies/ $\mu$ L	9500 (0–552,000)	342 (0–58,300)	NS

Values are given as median (min–max) or otherwise stated.

ART=antiretroviral treatment, HIV = human immunodeficiency virus, RPR, rapid plasma regain, NS=nonsignificant, VL=viral load.

**Table 2**  
**Characteristics of serofast state patients with and without neurosyphilis.**

	Neurosyphilis group (n=5)	No-neurosyphilis group (n=7)	P-value
Age (min–max), y	36 (29–53)	36 (26–54)	NS
RPR 12 mo after therapy (min–max)	128 (32–512)	8 (4–16)	.006
ART, n (%)	1 (20)	7 (100)	.005
CD4+ count (min–max), cells/ $\mu$ L	332 (188–551)	628 (312–1313)	.04
VL (min–max), copies/ $\mu$ L	94,950 (19,700–552,000)	204 (0–28,300)	.01
CSF-WBC (min–max), cells/ $\mu$ L	32 (24–127)	6 (3–9)	.003
CSF protein concentration (min–max), mg/dL	775 (429–1415)	547 (345–1098)	NS

Values are given as median (min–max) or otherwise stated.

ART=antiretroviral treatment, NS=nonsignificant, RPR=rapid plasma regain, VL=viral load.

patients. Lumbar punctures were performed in all of these patients. Based on the results of the CSF analysis, 5 cases (41.7%) of asymptomatic neurosyphilis were identified. Three patients had reactive CSF-VDRL. In the remaining 2, CSF-VDRL was nonreactive, but they had both CSF white blood count (CSF-WBC) of  $\geq 20/\mu$ L and elevated CSF protein concentration (i.e.,  $\geq 45$  mg/dL). The characteristics of individuals with and without neurosyphilis are shown in Table 2.

Patients with neurosyphilis were characterized by significantly higher RPR titer and higher viral load 12 months after syphilis treatment when compared to those without neurosyphilis (Table 2). Interestingly, all patients in whom neurosyphilis has been excluded (no-neurosyphilis group) were on ART. In contrast, only 1 patient from the neurosyphilis group (20%) was receiving antiretroviral treatment (in this patient HIV RNA levels and CD4+ count was 19,700 copies/ $\mu$ L and 551 cells/ $\mu$ L, respectively).

The multivariate logistic regression analysis revealed that not receiving ART independently increased the risk of neurosyphilis among HIV-infected patients who remained serofast after therapy of syphilis ( $R^2=0.7$ , odds ratio=4.5; 95% confidence interval 1.5–13.59,  $P=.003$ ).

#### 4. Discussion

Some previous studies evaluated the association of HIV status with serologic outcomes after treatment of syphilis by comparing patients with and without HIV infection.<sup>[9–12]</sup> However, results of these studies are inconsistent, and it remains unclear whether HIV-infected individuals are less likely to achieve a serologic cure. These conflicting results can be explained, in part, by the variability in the stages of syphilis and HIV infection status among patients included in these studies, and by an inconsistent definition of serologic nonresponse. More explicit seems to be that patients co-infected with HIV may have a significantly slower decrease in nontreponemal titer decrease than HIV-uninfected individuals.

In the present study, 6 and 12 months after completing syphilis therapy, 54.5% and 36.4% of patients (respectively) did not achieve a serologic cure (i.e., at least 4-fold decrease in RPR titer when compared to pretreatment values). When compared to previous, similarly designed studies which were dedicated to the assessment of serologic response among HIV-negative individuals, our findings seem to support the hypothesis of slower serologic response and higher frequency of serofast state after syphilis therapy in patients co-infected with HIV.<sup>[13–15]</sup>

In our study, the proportion of serologic responders significantly increased at 12 months after therapy when compared to this at 6 months (54.4% vs. 36.4%, respectively).

Such improvement of serologic treatment outcome has not been observed among HIV-negative individuals with syphilis. Thus, it may be reasonable to consider the diagnosis of serofast state in patients with HIV not at 6 but at 12 months after completing the treatment regimen.

To date, factors associated with serologic outcomes among HIV-infected persons with syphilis have been analyzed only in a limited number of studies. They found a CD4+ cell count of  $< 200$  cells/mL to be strongly correlated to serologic failure. It has been also estimated that ART can reduce the risk of serofast syphilis by more than 60%.<sup>[9]</sup> In the present study, we confirmed these previous findings. However, due to a small sample size, it was not possible to identify definite cut-off values for the CD4+ cells count that may be associated with higher risk of serologic failure. Similar to previous results, we found that ART reduced the risk of serofast state after treatment of syphilis by 65%.

Due to an inability to cultivate the causative agent of syphilis in vitro, most testing for syphilis treatment outcome relies on measurement of the immune responses. Untreated or inadequately treated syphilis can result in severe complications such as neurosyphilis or cardiovascular syphilis. Not surprisingly, considerable uncertainty exists as to whether the “serofast state” indicates persistent infection of *T pallidum* or a residual immune response in the absence of viable organisms. Only a few studies have been performed to measure the response to therapy following retreatment of serofast syphilis patients. They demonstrated only a minimal improvement in serologic response after retreatment with penicillin.<sup>[16,17]</sup> Therefore, it has been suggested that serofast state may be only a sign of persistent immune activation.

To the best of our knowledge, only 4 studies have performed lumbar punctures among individuals with serologic nonresponse to therapy of syphilis.<sup>[6–8,18]</sup> None of these studies were dedicated to HIV-infected patients with syphilis. In our study, we found high incidence of asymptomatic neurosyphilis (of 40%) among serofast syphilis patients who were co-infected with HIV. It should be highlighted that we used very strict criteria both for serologic failure and neurosyphilis. Additionally, we showed a significant association between the risk of neurosyphilis and lower CD4+ cell count, higher viral load and not-receiving ART at the posttreatment time point. In a multivariate analysis that included all above-mentioned variables, we found not-receiving ART to be independently associated with the risk of neurosyphilis among HIV-infected individuals who remained serofast after therapy of syphilis. Therefore, it seems that both CD4 cell reconstitution and reducing of HIV in the blood can have additive effect in the prevention of CNS involvement. On the other side, even when patients who receive ART do not experience CD4 cell increase, there are data to suggest that there are benefits to

outcome of syphilis treatment.<sup>[19]</sup> Thus, the impact of ART on the response of HIV-infected patients with syphilis cannot be simply explained by the CD4 cell reconstitution and reducing of viral load. We can only speculate that ART can have pleiotropic effects on the host immune response through mechanisms that have not been explained yet.

A number of studies have found the natural course of syphilis to be altered in HIV-infected patients.<sup>[20–22]</sup> Patients with HIV infection have well-described dysfunction of antibody formation what can result in less effective immune clearance of *T pallidum*.<sup>[23]</sup> The findings of our study raise uncertainty as to whether the serofast state in HIV-infected individuals is or is not indicative of persistence of treponema since as many as 40% of serofast patients had neurosyphilis. Further studies are needed to investigate the clinical significance of the serofast state after syphilis therapy among patients co-infected with HIV.

The small sample size and enrollment of patients only with secondary and early latent syphilis reduces the generalizability of our results. Despite an increasing number of cases worldwide, syphilis remains a relatively rare disease in developed countries (up to 3.5 cases/100,000 per year in Poland). Thus, further multi-center studies are needed.

In conclusion, we showed for the 1st time a high frequency of asymptomatic neurosyphilis among HIV-infected individuals who remain serofast after receiving the recommended therapy for syphilis. Our results support the hypothesis that serologic failure in this group can be, in a significant proportion of patients, related to persistence of spirochetes. If left untreated, asymptomatic neurosyphilis may progress to the symptomatic phase and result in severe neurologic and mental impairment. Thus, based on these findings, we propose that CSF examination should be considered in every HIV-infected patient who did not achieve proper serologic response after syphilis therapy. Serofast patients who are not receiving ART seem to be at particular risk of neurosyphilis.

## Author contributions

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