7

Serologic Response to Treatment in Syphilis

Neuza Satomi Sato
Center of Immunology, Institute Adolfo Lutz
São Paulo, SP
Brazil

1. Introduction

Serologic monitoring is the way to determine adequate treatment response, which is typically defined as a fourfold decline in Rapid Plasma Reagin (RPR) or Veneral Disease Research Laboratory (VDRL) titer within 6 months of treatment for patients with primary or secondary syphilis and within 12 months of treatment for patients with early latent syphilis. Studies documented close to 100% rate seroreversion 1 -2 years after penicillin treatment, depending on the stage of syphilis and duration of symptoms.

HIV co-infection has several effects on the presentation, diagnosis, disease progression, and therapy of syphilis. Unusual syphilis serologic titers have been reported in HIV-infected patients, with either unexpected high or low titer, as well as delayed serologic response at the time of diagnosis. There are several studies concerned to syphilis serological treatment response comparing HIV-infected and HIV-negative patients. Some studies reported a slower serological response in HIV infected patients. A recent study showed that the use of high active antiretroviral therapy (HAART) and the routine use of macrolides for the prevention of opportunistic infections may reduce syphilis serologic failure rates among HIV-infected patients who have syphilis.

Penicillin G, administered parenterally, is the preferred drug for treating all stages of syphilis (French et al., 2009; Workowski & Berman, 2010). Treatment failure can occur with any regimen. Assessing response to treatment frequently is difficult, and definitive criteria for cure or failure have not been established.

According to the CDC guidelines recommendations, clinical and serologic evaluation should be performed 6 months and 12 months after treatment of patients with primary or secondary syphilis; more frequent evaluation might be prudent if follow-up is uncertain.

Patients who have signs or symptoms that persists or recurs or who have sustained fourfold increase in nontreponemal test titer probably failed treatment or were reinfected. The latent syphilis is not transmitted sexually; the objective of treating patients with this stage of disease is to prevent complication. Quantitative nontreponemal serologic tests should be repeated at 6, 12, and 24 months for follow- up. Limited information is available concerning clinical and serological follow-up of patients who have tertiary syphilis. HIV-infected persons should be evaluated clinically and serologically for treatment failure at 3, 6, 9, 12, and 24 months after therapy (Workowski & Berman, 2010).

Usually, treatment failure cannot be distinguished from reinfection with *T. pallidum*; in these cases a CSF analysis is recommended.

2. Serorevertion of the serological tests for syphilis

Patients treated for syphilis are monitored by quantitative nontreponemal tests (VDRL or RPR). The rate of decrease of serologic titers is influenced by many factors, including the history of previous syphilis, the stage of infection, the baseline serologic titers, the immune status, and the administered treatment (Augenbraun et al., 1998; Brown et al., 1999; Fiumara, 1977a, 1977b, 1978; Ghanem et al., 2007; Romanowski et al., 1987; Talwar et al., 1992).

2.1 Nontreponemal antibodies

Nontreponemal test antibody titers may correlate with disease activity, and results should be reported quantitatively. Nontreponemal test titers usually declines after treatment and might become nonreactive with time, however, in some persons, these antibodies can persist for a long period of time, a response referred to as the serofast reaction (Workowski & Berman, 2010).

In a study of 586 patients with early syphilis treated with benzathine penicillin, 2.4 mU, the VDRL was nonreactive in 97% of patients with primary syphilis and 77% with secondary syphilis, within 2 years (Schroeter et al., 1972).

Fiumara carried a series of studies of serologic response to treatment in patients at different stage of syphilis disease. A study of 588 patients with primary syphilis found that all patients were seronegative after 1 year, and all 623 with secondary syphilis had seroreverted their nontreponemal tests within within 2 years. A study which included 275 early latent syphilis patients of less than one year duration, demonstrated that all but 2 of the patients became seronegative within 4 years. In another study of 123 patients with late latent syphilis, 44% became seronegative within five years, and 56% had persistently positive nontreponemal tests (Fiumara, 1979). Most of the patients in these studies were treated with higher dose of penicillin than currently recommended (Workowski & Berman, 2010), and the rest of the patients were treated with tetracycline.

A study of serologic response in a cohort of 818 patients treated for primary or secondary syphilis found that VDRL titer declined approximately fourfold at 3 months and eightfold at 6 months. The serological response of patients treated with erythromycin was inferior to that achieved by penicillin or tetracycline (Brown et al., 1999).

Anderson et al. reported that in most patients the VDRL test showed a consistent fall in titer after treatment, however a small proportion continued to give positive results with no evidence of reinfection or treatment fail (Anderson et al., 1989). The study analyzed data from 946 patients with primary and 854 patients with secondary syphilis, and follow-up serology by VDRL was carried at 6 and 18 months. Of the patients with primary syphilis, seroreversion were found in 70% and 85%, respectively at 6 and 18 months. Patients with secondary syphilis, 45% became seronegative within 6 months and 68% at 18 months. Treatment most often used was intramuscular procaine penicillin (> 85%), benzathine penicillin was used in only approximately 3% of patients, and non-penicillin drugs included erythromycin or tetracycline. No significant differences were found comparing titers in patients treated with penicillin or non-penicillin (Anderson et al., 1989).

The serologic response was evaluated in a historical cohort study of patients treated for syphilis from 1981 to 1987 in Alberta, Canada. A total of 882 patients included in the analyses were treated with the currently recommended benzathine penicillin regimens. After 3 years, 72% and 56% of patients with initial primary and secondary syphilis had

seroreversal of RPR tests. A fourfold decrease and a sixfold decrease were seen in patients with primary and secondary syphilis by 6 and 12 months, respectively, while a fourfold decline was seen in patients with early latent syphilis by 12 months. Serologic response was not affected by gender, age, race, or sexual orientation. Patients with their first infection were more likely to experience RPR seroreversion than those with repeat infections (Singh & Romanowski, 1999).

In a retrospective study of 1,532 patients with early syphilis, the majority of seropositive cases had nonreactive VDRL by 6 months after treatment. Seroreversion was observed in 84% of patients with primary syphilis, 72% of patients with secondary syphilis, and 81% of patients with early latent syphilis by 6 months. The percentages were 93, 92, and 88%, respectively at the end of the 30 months study period. Approximately 86% of patients were treated with benzathine penicillin, 2.4 mU. Others were treated with higher doses of benzathine penicillin, 4.8 mU, and procaine penicillin, which appeared to accelerate the speed of seroconversion (Talwar et al., 1992).

2.2 Anti-treponemal antibodies

Treponemal test antibody titers should not be used to assess treatment response. Most patients who have reactive treponemal tests will have reactive tests for the reminder of their lives, regardless of treatment or disease activity.

However, the disappearance of reactivity in the treponemal tests after treatment of immunocompetent patients and over time has been reported. Schoeter et al. reported that 14% of patients with early syphilis lost their reactivity in the FTA-abs test within 2 years after treatment (Schroeter et al., 1972). Another study demonstrated that in patients with first episode syphilis, 24% of had a non-reactive FTA-abs and 13% had a nonreactive MHA-Tp in 3 years (Romanowski et al., 1991). A prospective, cohort treatment study of 261 patients with early syphilis that had 1 year serologic follow-up with FTA-abs or MHA-TP found seroreversion in 9% and 5% of cases, respectively. No association between HIV-seropositivity and treponemal specific test seroreversion was demonstrated (Augenbraun et al., 1998). Castro et al. performed a study of serologic follow-up of small number of patients with early syphilis. Of the 54 patients evaluated 6 months after therapy, 70% had at least twofold titer decrease when using the specific passive agglutination test (TP.PA); only 22 patients returned for 12 months evaluation, and 19 (86%) had decreased antibody titer. Seroreversion for anti-treponemal antibodies were not found in this study (Castro et al., 2001).

Other studies were performed by Western Blot technique, which provides analyses of reactivity against each specific antigenic fraction. Kim et al. observed a significant loss of anti-Tp47 after treatment of primary syphilis, with complete seroreversion in 11 months (Kim et al., 1989). George group studied 124 persons with clinically diagnosed syphilis by using densitometric quantization and spreadsheet normalization to refine the parameters defining treponemal WB for syphilis. The reactivity against Tp47 was 100% before treatment, while 28% had lost anti-Tp47 over 12 months after treatment (Fraser et al., 1998). More studies are necessary to state if a specific treponemal protein can be used as a potential marker for monitoring treatment.

2.3 Specific IgM

For long time, the utility of IgM in the diagnosis of syphilis has been discussed. Although there have been multiple studies addressing the use of IgM, the results have conflicted

somewhat. In the past, a study for quantitative evaluation of the FTA-abs-IgM and VDRL in treated and untreated syphilis revealed sera remained reactive with increased titers for more than one year after treatment in 19.5% patients with primary and 15% patients with secondary syphilis. In follow-up of nine patients with secondary syphilis, FTA-abs-IgM and VDRL titers showed only partial agreement during the course of observation. The FTA-abs-IgM titer usually reverted to non-reactivity later than de VDRL dilutions, indicating that VDRL was better for monitoring treatment (Luger et al., 1977).

Actually, the results of specific IgM tests, either by Western Blot or ELISA for capture of IgM, are essential in the diagnosis of congenital syphilis as well as in the recognition of re-infection; they indicate the need for treatment and are useful in the assessment of the effectiveness of therapy (Schmidt et al., 1994).

McMillan & Young analyzed reactivity in VDRL test and Mercia IgM-Elisa (IgM-EIA) after treatment of 229 patients with early syphilis. The seroreversion observed for IgM-EIA and VDRL were respectively 62% and 41%, at 3 months follow-up, and 1 year after treatment the IgM-EIA were negative in 92% patients while VDRL were negative in 70% cases. A fourfold or greater decrease in VDRL titer occurred in 99% of patient at 3 and 12 months. It was concluded that the Mercia IgM EIA is as sensitive as VDRL in monitoring treatment of primary syphilis, but not as sensitive as the finding of a fourfold or eightfold decrease in VDRL titer in patients treated for secondary or early latent infection (McMillan & Young, 2008).

A study to evaluate *Treponema pallidum*-specific IgM as marker of infectious syphilis in human immunodeficiency virus (HIV)-infected patients was performed with 20 samples from HIV-infected patients with untreated syphilis and follow-up at 3, 6 or 12 months after treatment. The IgM detection detection by Mercia-EIA appears to be a reliable marker for untreated syphilis in HIV-infected patients with primary or secondary syphilis. After treatment, IgM was no longer detected after three months in the majority of patients (87%) and was either negative or equivocal in all patients after six and 12 months (Rotty et al., 2010). It has also been suggested that detection of syphilis specific IgM may correlate with active disease and assist in differentiating active from past and successfully treated syphilis.

3. Syphilis in HIV-infected patients

For most HIV-infected persons, serologic tests are accurate and reliable for the diagnosis of syphilis and for following a patient's response to treatment. However, atypical syphilis serologic tests results can occur in HIV-infected persons. Most reports have involved serologic titers that were higher than expected, but false-negative and delayed appearance of seroreactivity or fluctuating titers also have been reported (Palmer et al., 2003).

When serologic test do not correspond with clinical findings suggestive of early syphilis, use of others tests should be considered, such as biopsy, darkfield microscopy or polymerase chain reaction.

Besides studies of syphilis serologic response rate, several authors have evaluated the time to serologic response according to HIV status, with conflicting results. Some studies found a slower serologic response in HIV infected patients, but other did not. Also, a higher risk of serological failure was associated with HIV infection (Ghanem et al., 2007; Gonzalez-Lopez et al., 2009; Kofoed et al., 2006; Malone et al., 1995; Telzak et al., 1991; Walter et al., 2006; Yinnon et al., 1996).

Neurosyphilis should be considered in the differential diagnosis of neurological disease in HIV-infected person. For patients whose nontreponemal test titers do not decrease fourfold within 6-12 months therapy, CSF examination and retreatment also should be strongly considered (Workowski & Berman, 2010).

3.1 Pre-HAART

According to CDC guidelines, HIV infected person should be evaluated clinically and serologically for treatment failure at 3, 6, 9, 12 and 24 months. If after therapy, nontreponemal titers do not decline fourfold during 12-24 months or titers rise fourfold, at any time, it might be indicative of treatment failure. A higher risk of serological failure was associated with HIV infection.

Telzak et al. performed a retrospective review of response to standard therapy in HIV-infected patients with primary or secondary syphilis. They reported that in patients with primary syphilis, HIV infected patients were less likely to have a fourfold or greater RPR decrease or seroreversion within 6 months of treatment compared with HIV negative patients ($P = 0.03$). Patients with secondary syphilis had similar serological response after treatment, regardless of HIV status (Telzak et al., 1991).

Malone et al reported a relapse or failure rate of 18% in 56 HIV positive patients with follow-up for a mean of 28 months. Relapse occurred more than 12 months after initial therapy in 6 of 10 patients (60%) who experienced relapse; 5 patients experienced multiple relapses. Treatment failure was not related with CD4 count or to a specific antimicrobial regimen. However, the results suggest that patients with clinical evidence of secondary syphilis or with reactive cerebrospinal fluid VDRL test titers were at highest risk of subsequent relapse or treatment failure when monitored for an average of 2 years (Malone et al., 1995).

Rolfs et al. performed a study of randomized trial of enhanced therapy for early syphilis in 541 patients including 101 patients who had HIV infection. Patients were treated with 2.4 mU benzathine penicillin G with or without enhanced therapy consisting of 2 g of amoxicillin and 500 mg of probenecid 3 times daily for 10 days. After 1 year follow-up, 14% of patients were serologically defined as treatment failures; the serologic failure rate was higher in HIV infected patients. Enhanced treatment with amoxicillin and probenecid did not improve the outcomes, and the authors concluded that CDC recommendations for treating early syphilis are adequate for most patients, whether or not they have HIV infection (Augenbraun et al., 1998).

Ghanem and collaborators performed a comparative study of serological response to syphilis treatment in 129 HIV positive and 168 HIV negative patients attending sexually transmitted diseases clinics between 1992 and 2000. Serologic failure was defined as lack of a fourfold drop in RPR titer by 400 days after treatment or a fourfold increase titer between 30 and 400 days. They found 22 serologic failure (17%) in HIV positive groups and 5 in HIV negative group ($p < 0.001$). The median times to successful serological responses were 278 and 126 days, respectively for HIV positive and HIV negative groups ($p < 0.001$). The difference in serologic failure rates was significant in the subgroup of early syphilis, while the difference in median times to successful serologic response was also significant in the subgroup of late latent syphilis. A higher risk of serologic failure was associated with HIV infection (Ghanem et al., 2007).

A study of 41 cases of syphilis diagnosed in HIV-1 infected patients revealed treatment failure in 10% (4 patients) in 7 months follow-up. Also, syphilis was associated with decrease in CD4 cell count and an increase in HIV-RNA levels, and both improved after syphilis treatment (Kofoed et al., 2006). The evaluation of 64 HIV seropositive patients and matched controls retrieved a slower serologic response in HIV infected patients. The HIV-positive patients with initial RPR less than 1:32 experienced a significantly slower decrease in RPR at 12 months than did the controls ($P < .001$) (Yinnon et al., 1996).

Another study by Agmon-Levin et al. analyzed response to recommended treatments of 81 patients which completed 12 months follow-up. Treatment success was documented in 26%, a high rate of serofast (41%) and treatment failure (33%) were found. The immune response correlated with immune status of the patients, the mean CD4 counts were higher and HIV viral load were lower in patients with successful treatments (Agmon-Levin et al., 2010). The increased prevalence of serofast reaction has been reported in co-infected patients previously. This might be attributed to HIV associated hyperglobulinaemia or a longer period of time (2-5 years) required for VDRL levels to decline in HIV infected patients, especially those with prolonged infection or low VDRL levels, lower than 1:8 (Manavi & McMillan, 2007).

González-López et al. performed a longitudinal, retrospective study in a cohort of HIV positive and HIV negative patients with syphilis. Serologic failure was observed in 29.6% (37/125) of HIV positive patients and 11.2% (7/62) HIV negative patients (odds ratio, 3.3; $p < 0.05$). A slower serologic response to treatment was demonstrated in men HIV infected patients with late stage syphilis. HIV negative patients responded more frequently to treatment, but after 2 years follow-up, both groups shared similar response rates (Gonzalez-Lopez et al., 2009). Another study found that HIV infected patients had greater rate of incident syphilis compared with HIV uninfected. Also, HIV infected patients had a greater likelihood to decline in RPR test titer and serologic failure (Horberg et al., 2010).

Others reported no association between serologic failure for syphilis treatment and patient's HIV status. Manavi et al. performed a study to compare outcome of syphilis treatment in HIV infected and uninfected patients. Patients with diagnosis of syphilis who had 24 months follow-up syphilis serology included 161 HIV negative and 129 HIV positive patients. The lack fourfold decrease of VDRL test titer within 12 months in absence of history of re-infection was considered as treatment failure. After 12 months, 63% of HIV negative and 70% of HIV positive patients were treated ($p = 0.04$). HIV serologic status were not associated with success of treatment, and treatment failure in a proportion of HIV positive patients was due to slower decline in VDRL titer rather than lack of response to treatment (Manavi & McMillan, 2007).

Seroreversion of reactive antibodies in patients previously treated for syphilis have been reported in patients with AIDS (Haas et al., 1990; Johnson et al., 1991). Haas analyzed sera from 90 HIV positive and 19 HIV negative men observed for a mean follow-up of 4 years. None of the HIV seronegative individuals lost reactivity to a treponemal test, whereas 7% of the seropositive asymptomatic individuals and 38% of those with symptomatic HIV infection had loss of reactivity. Symptomatic HIV infection was associated with loss of reactivity, as a CD4 count less than 200 cells/uL, a CD4/CD8 ratio less than 0.6, a single prior episode of syphilis, and a low VDRL titer at the time of the last documented episode of syphilis (Haas et al., 1990). Johnson found that 10% (3/29) patients with AIDS had loss of reactivity for both the hemagglutination (TPHA) and FTA-abs over a period of 3 years;

whereas no seroreversion was observed in the 29 controls (Johnson et al., 1991). Janier et al evaluated the long-term outcome of syphilis treponemal tests in a cohort of HIV positive male homosexuals with a history of treated syphilis (69 patients) as compared with HIV negative controls (49 patients). The decrease in VDRL titers was not different between 2 groups ($p = 0.053$). Time to seroreversion was shorter in HIV positive patients for TPHA ($p = 0.009$) and FTA-abs test ($p = 0.001$). The seroreversion of the FTA-abs test was related to a low baseline CD4 cell count ($p = 0.003$), while the seroreversion of TPHA and VDRL were not related. After adjustment for the CD4 cell count, only TPHA titer had significant decrease and seroreversion in HIV positive patients (French et al., 2009).
However, another study found no association between HIV seropositivity and seroreversion of treponemal antibodies, in patients treated for early syphilis (Augenbraun et al., 1998). Also, seroreversion of treponemal antibodies had been reported in immunocompetent patients treated for early syphilis (Schroeter et al., 1972) or first episode of syphilis (Romanowski et al., 1991).

3.2 Post-HAART

Antiretroviral therapy significantly reduced the time to achieve response to syphilis treatment in HIV-positive patients (Gonzalez-Lopez et al., 2009).
Farhi et al. evaluated the effect of HIV on clinical and serologic features of syphilis at baseline and during follow-up in the pos-HAART era, in a retrospective cohort study of patients with syphilis treated according to the European guidelines. Serologic failure was defined as either a fourfold rise in VDRL titers 30-400 days after treatment or a lack of fourfold drop in VDRL titers at 270-400 days post treatment. Among 144 informative syphilis cases, a lower rate of serologic response was observed in HIV infected patients, however this difference was not significant (91.8% vs. 98.3%, $p = 0.14$). Also, a median delay to serologic response was similar in both group of patients ($p = 0.44$), HIV positive (117 days) and in HIV negative (123 days). Serologic failure was significantly associated with a history of previous syphilis ($p < 0.05$). The authors concluded that effect of HIV on serologic response to syphilis treatment is minimal or absent for patients under HAART treatment (Farhi et al., 2009; Farhi & Dupin, 2010). Another study evaluated whether the use of HAART impact syphilis serologic responses. Serologic failure was defined as the lack of fourfold decrease in RPR titers 9 – 12 months after therapy or a fourfold increase in titers a month or later after therapy. A total of 71 cases among 180 patients with syphilis presented serologic failure, and the median follow-up time was 5.3 years. CD4 cells count of < 200 cells/mL at the time of syphilis diagnosis was associated with an increased risk of serologic failure. The use of HAART was associated with 60% reduction in the rate of serologic failure, independent of concomitant CD4 cell response (Ghanem et al., 2008).

4. Neurosyphilis

Central neural system (CNS) involvements can occur during any stage of syphilis. CSF laboratory abnormalities are common in persons with early syphilis, even in the absence of clinical neurological findings. A CSF examination should be performed when clinical evidence of neurologic involvement is observed, including ocular manifestations frequently associated with neurosyphilis. There is no gold standard test to diagnose neurosyphilis. According to the diagnostic criteria, neurosyphilis can be defined in two categories,

confirmed and presumptive, both can occur at any stage of syphilis. Confirmed neurosyphilis presents reactive CSF VDRL, while presumptive neurosyphilis is defined when patients present clinical signs or symptoms consistent with syphilis without an alternate diagnosis to account for these, non reactive CSF VDRL and CSF pleocytosis or elevated protein (Ghanem, 2010).

A positive VDRL results establishes a diagnosis of neurosyphilis, but negative VDRL does not exclude it. The use of CSF RPR is not currently recommended, because RPR is less specific in CSF. The CSF FTA-abs test is less specific than the VDRL for diagnosis of neurosyphilis, but it is highly sensitive. A negative CSF FTA-abs test excludes the diagnosis of neurosyphilis (Workowski & Berman, 2010).

An alternative CSF tests to diagnose neurosyphilis in HIV infected patients had been proposed when the CSF VDRL is nonreactive. The combination of the CSF FTA-abs and assessment of CSF B cells can be used to identify syphilis patients with and without neurosyphilis when the CSF VDRL is non reactive (Marra et al., 2004c).

According to the European guidelines, a patient should be treated as for neurosyphilis in the following conditions: reactive CSF VDRL and treponemal antibody tests (TP hemagglutination assay or FTA-abs), WBC-CSF exceeds 10/mm^3 and IgG index is 0.70 or higher or the IgM index is 0.10 or higher in CSF (Goh & van Voorst Vader, 2001).

A study by Marra et al demonstrated that a serum RPR titer ≥ 1:32 is predictive of neurosyphilis in all patients with syphilis and that a peripheral blood CD4 cell count ≤ 350 cell/uL is an additional risk factor for neurosyphilis in HIV infected patients. Also, the risk associated with these parameters is independent of previous syphilis therapy and stage of syphilis (Marra et al., 2004a).

If CSF pleocytosis was present initially, a CSF examination should be repeated every 6 months until the cell count turn to normal (Workowski & Berman, 2010).

The success of therapy in patients with symptomatic neurosyphilis is assessed by resolution of symptoms and signs, and normalization of CSF abnormalities, including pleocytosis, elevated protein concentration or a reactive CSF VDRL test. If neurosyphilis is asymptomatic, normalization of CSF measures is the only means of assessing treatment success (Workowski & Berman, 2010). After therapy, the changes in CSF VDRL or CSF protein occur more slowly than cell counts, and persistent abnormalities might be less important. The leukocyte count is a sensitive measure of the effectiveness of therapy (Marra et al., 2000; Marra et al., 2004b).

Resolution of all serum and CSF abnormalities were resolved by 30 weeks in most patients not infected with HIV (Marra et al., 1996). Another study by the same group found that in most instances, normalization of serum RPR titer correctly predicts normalization of CSF and clinical measures after neurosyphilis treatment and follow-up lumbar puncture can be avoided. However, using the serum RPR criteria, 12-37% of individuals can be misclassified as experiencing treatment success, and among HIV infected patients, misclassification is most common in those not receiving anti-retroviral therapy (Marra et al., 2008). A study found that resolution of CSF abnormalities was slower in patients infected with HIV after treatment of neurosyphilis (Marra et al., 1996), particularly if the peripheral blood CD4 cells count is lower than 200 cell/uL (Marra et al., 2004c).

A study of neurosyphilis in a clinical cohort of HIV-1 infected patients found that HAART therapy to reverse immunosupression may help mitigate neurological complication of syphilis. In this cohort, the degree of immunosuppression, as measured by CD4 cell count,

was an independent risk factor for developing neurosyphilis, and the use of HAART reduced the odds of neurosyphilis by 65%. Among patients diagnosed and treated for neurosyphilis, there was a trend for decreased risk of serological failure in patients who received six or more months of HAART therapy during a median follow-up of 4.3 years (Ghanem et al., 2008).

5. Syphilis during pregnancy and congenital syphilis

All women should be screened serologically for syphilis early in pregnancy. Serologic testing should be performed at 28-32 weeks' gestation and repeated at delivery. Also, any woman who delivers a stillborn infant after 20 weeks' gestation should be tested for syphilis. Serologic titers can be checked monthly in women at high risk for reinfection or in geographic areas in which the prevalence of syphilis is high. If serological screening was performed by treponemal antibody testing, pregnant with reactive results for treponemal antibodies should have confirmatory testing with nontreponemal tests with titers (Workowski & Berman, 2010).

Transplacental infection can occur at any stage pregnancy (Wicher & Wicher, 2001). Most cases of congenital syphilis occur as a result of a failure to detect and treat syphilis in pregnant women. The failure of treatment and congenital syphilis has been reported (Lasfargue et al., 2009; Marangoni et al., 2008).

Regardless of the regimen used to treat syphilis during pregnancy, clinicians should recognize the possibility of occasional treatment failures and the importance of adequate follow-up of infants at risk for congenital syphilis (Conover et al., 1998).

Factors that contribute to treatment failure include maternal stage of syphilis (early stage syphilis), advancing gestational age at treatment, higher VDRL titers at treatment and delivery, and a short interval from treatment to delivery, defined as ≤30 days, and these factors can be used to target neonates at high risk for congenital syphilis (Sheffield et al., 2002).

The diagnosis of congenital syphilis is based both, on a clinical evaluation and on laboratory investigations. Diagnosis is complicated because more than half of all infants are asymptomatic at birth, and sign in symptomatic infants may be subtle and nonspecific.

All infant born to women who have reactive serologic test for syphilis should be examined thoroughly for evidence for congenital syphilis, darkfield microscopy examination of suspicious lesions or body fluids also should be performed. Pathologic examination of the placenta or umbilical cord using specific fluorescent anti-treponemal antibody staining is suggested. Also, infants born to mothers who have reactive nontreponemal and treponemal test results should be evaluated with quantitative nontreponemal serological test (VDRL or RPR) performed on infant serum. It is not necessary to conduct a treponemal test on a newborn's serum, however a test to detect immunoglobulin IgM can be recommended (Workowski & Berman, 2010). The detection of specific IgM is currently the most sensitive serological method, and the presence of specific IgM should be considered as evidence of a congenital *T. pallidum* infection (Herremans et al., 2010).

In the past, a retrospective analysis of the serologic response to treatment of syphilis during pregnancy was performed. Treatment response was evaluated by comparing each post-treatment titer of a patient to her pre-treatment titer, and it was classified as a positive response (≥ fourfold titer decline) or a negative response (< fourfold titer decline). A

positive response following treatment was significant more likely if there was no prior history of syphilis or if there was a high initial RPR titer (> 32). Only 61% (33/54) had positive response at or greater than 3 months observations. The study revealed that an absence of a history of syphilis and an initial high RPR titer are predictive of a positive response following appropriate treatment (Galan et al., 1997).

Chang et al. performed a retrospective survey to determine the time of seroreversion of serological tests for syphilis in 52 uninfected newborn to mothers who were adequately treated for syphilis. Most seropositive untreated newborns became seronegative within 6 months after birth for the VDRL and within 1 year for the TPHA and FTAabs. However, 3 infants showed persistently positive VDRL and TPHA tests. The VDRL seroreversion were documented at 9 and 10 months after birth, and TPHA remained positive over 12 months. These infants had no clinical evidences of congenital syphilis and presented nonreactive 19S-IgM- FTAabs (Chang et al., 1995).

All seroreactive infants and infants whose mother were seroreactive at delivery should receive careful follow-up examinations and serologic nontreponemal test every 2-3 months Until the test become nonreactive or the titer has decreased fourfold. If the infant was adequately treated or was not infected, nontreponemal antibody titers should decline by age 3 months and should be nonreactive by age 6 months. The serologic response after therapy might be slower for infants treated after the neonatal period. (Workowski & Berman, 2010). Passively transferred maternal treponemal antibodies can be present in an infant until the age 15 months; therefore, a reactive treponemal test after age 18 months is diagnostic of congenital syphilis. Infants whose initial CSF evaluations are abnormal should undergo a repeat lumbar puncture approximately every 6 months until the results are normal (Workowski & Berman, 2010).

6. Potential markers

A specific anti-treponemal IgM-EIA may be helpful in monitoring the serological response to treatment in RPR/VDRL negative primary syphilis (French et al., 2009).

Recently, a study examined the relationship between neurosyphilis and CSF concentration of CXCL13 in HIV infected patients with syphilis. CXCL13 is a B cell chemoattractant chemokine (C-X-C motif) ligant 13. They found that, compared to patients with uncomplicated syphilis, CSF CXCL13 concentration is significantly higher in patients with both asymptomatic and symptomatic neurosyphilis, and CSF CXCL13 concentration declines after neurosyphilis treatment (Marra et al., 2010). This may be particularly useful marker for diagnosis and treatment response evaluation of neurosyphilis in HIV infected patients (Marra et al., 2010).

7. Conclusion

There is no direct microbiologic test of cure of syphilis disease. Treatment response in current practice can be defined clinically and/or serologically by regular follow-up of quantitative serologic test for nontreponemal antibody (VDRL or RPR). Also, it is difficult to distinguish relapse from reinfection. The specific IgM antibodies may be helpful in this issue. The most relevant markers of treatment response are essential to improve the follow-up accuracy. Current research should focus on stronger efficacy assessment tool than the actual nontreponemal serology in use. The search for direct *T. pallidum* identification methods such as exploring molecular marker by *T.pallidum* specific polymerase chain reaction may be a promising approach.

8. References

Agmon-Levin, N., Elbirt, D., Asher, I., Gradestein, S., Werner, B. & Sthoeger, Z. (2010). Syphilis and HIV co-infection in an Israeli HIV clinic: incidence and outcome. *International Journal of Std & Aids* Vol.21, No.4, (April 2010), pp. 249-252, ISSN 1758-1052

Anderson, J., Mindel, A., Tovey, S. J. & Williams, P. (1989). Primary and secondary syphilis, 20 years' experience. 3: Diagnosis, treatment, and follow up. *Genitourinary Medicine* Vol.65, No.4, (August 1989), pp. 239-243, ISSN 0266-4348

Augenbraun, M., Rolfs, R., Johnson, R., Joesoef, R. & Pope, V. (1998). Treponemal specific tests for the serodiagnosis of syphilis. Syphilis and HIV Study Group. *Sexually Transmitted Disease* Vol.25, No.10, (November 1998), pp. 549-552, ISSN 0148-5717

Brown, T. J., Yen-Moore, A. & Tyring, S. K. (1999). An overview of sexually transmitted diseases. Part I. *Journal of the American Academy of Dermatology* Vol.41, No.4, (October 1999), pp. 511-529, ISSN 0190-9622

Castro, R. R., Prieto, E. S., Santo, I., Azevedo, J. & Exposto, F. d. L. (2001). Evaluation of the Passive Particle Agglutination Test in the Serodiagnosis and Follow-up of Syphilis. *American Journal of Clinical Pathology* Vol.116, No.4, (October 2001), pp. 581-585, ISSN 1943-7722

Chang, S. N., Chung, K. Y., Lee, M. G. & Lee, J. B. (1995). Seroreversion of the serological tests for syphilis in the newborns born to treated syphilitic mothers. *Genitourinary Medicine* Vol.71, No.2, (April 1995), pp. 68-70, ISSN 0266-4348

Conover, C. S., Rend, C. A., Miller, G. B., Jr. & Schmid, G. P. (1998). Congenital syphilis after treatment of maternal syphilis with a penicillin regimen exceeding CDC guidelines. *Infectious diseases in obstetrics and gynecology* Vol.6, No.3, pp. 134-137, ISSN 1064-7449

Farhi, D., Benhaddou, N., Grange, P., Zizi, N., Deleuze, J., Morini, J. P., Gerhardt, P., Krivine, A., Avril, M. F. & Dupin, N. (2009). Clinical and serologic baseline and follow-up features of syphilis according to HIV status in the post-HAART era. *Medicine (Baltimore)* Vol.88, No.6, (November 2009), pp. 331-340, ISSN 1536-5964

Farhi, D. & Dupin, N. (2010). Management of syphilis in the HIV-infected patient: Facts and controversies. *Clinics in Dermatology* Vol.28, No.5, (August 2010), pp. 539-545, ISSN 0738-081X

Fiumara, N. J. (1977a). Treatment of secondary syphilis: an evaluation of 204 patients. *Sexually Transmitted Diseases* Vol.4, No.3, (July 1977), pp. 96-99, ISSN 0148-5717

Fiumara, N. J. (1977b). Treatment of seropositive primary syphilis: an evaluation of 196 patients. *Sexually Transmitted Diseases* Vol.4, No.3, (July 1977), pp. 92-95, ISSN 0148-5717

Fiumara, N. J. (1978). Treatment of early latent syphilis of less than a year's duration: an evaluation of 275 cases. *Sexually Transmitted Diseases* Vol.5, No.3, (Jul 1978), pp. 85-88, ISSN 0148-5717

Fiumara, N. J. (1979). Serologic responses to treatment of 128 patients with late latent syphilis. *Sexually Transmitted Diseases* Vol.6, No.4, (October 1979), pp. 243-246, ISSN 0148-5717

Fraser, C. M., Norris, S. J., Weinstock, G. M., White, O., Sutton, G. G., Dodson, R., Gwinn, M., Hickey, E. K., Clayton, R., Ketchum, K. A., Sodergren, E., Hardham, J. M., McLeod, M. P., Salzberg, S., Peterson, J., Khalak, H., Richardson, D., Howell, J. K., Chidambaram, M., Utterback, T., McDonald, L., Artiach, P., Bowman, C., Cotton, M. D., Fujii, C., Garland, S., Hatch, B., Horst, K., Roberts, K., Sandusky, M.,

Weidman, J., Smith, H. O. & Venter, J. C. (1998). Complete Genome Sequence of Treponema pallidum, the Syphilis Spirochete. *Science* Vol.281, No.5375, (July 1998), pp. 375-388, ISSN 0036-8075

French, P., Gomberg, M., Janier, M., Schmidt, B., van Voorst Vader, P. & Young, H. (2009). IUSTI: 2008 European Guidelines on the Management of Syphilis. *International Journal of Std & Aids* Vol.20, No.5, (May 2009), pp. 300-309, ISSN 0956-4624

Galan, H. L., Montalvo, J. F. & Deaver, J. (1997). Retrospective analysis of the serologic response to the treatment of syphilis during pregnancy. *Infectious diseases in obstetrics and gynecology* Vol.5, No.1, pp. 23-28, ISSN 1064-7449

Ghanem, K. G. (2010). Neurosyphilis: A Historical Perspective and Review. *CNS Neuroscience & Therapeutics* Vol.16, No.5, (July 2010), pp. e157-e168, ISSN 1755-5949

Ghanem, K. G., Erbelding, E. J., Wiener, Z. S. & Rompalo, A. M. (2007). Serological response to syphilis treatment in HIV-positive and HIV-negative patients attending sexually transmitted diseases clinics. *Sexually Transmitted Infections* Vol.83, No.2, (April 2007), pp. 97-101, ISSN 1368-4973

Ghanem, K. G., Moore, R. D., Rompalo, A. M., Erbelding, E. J., Zenilman, J. M. & Gebo, K. A. (2008). Antiretroviral Therapy Is Associated with Reduced Serologic Failure Rates for Syphilis among HIV-Infected Patients. *Clinical Infectious Diseases* Vol.47, No.2, (July 2008), pp. 258-265, ISSN 1537-6591

Goh, B. T. & van Voorst Vader, P. C. (2001). European guideline for the management of syphilis. *International Journal of STD & AIDS* Vol.12, No.suppl_2, (October 2001), pp. 14-26, ISSN 0956-4624

Gonzalez-Lopez, J. J., Guerrero, M. L., Lujan, R., Tostado, S. F., de Gorgolas, M. & Requena, L. (2009). Factors determining serologic response to treatment in patients with syphilis. *Clinical Infectious Diseases* Vol.49, No.10, (November 2009), pp. 1505-1511, ISSN 1537-6591

Haas, J. S., Bolan, G., Larsen, S. A., Clement, M. J., Bacchetti, P. & Moss, A. R. (1990). Sensitivity of Treponemal Tests for Detecting Prior Treated Syphilis during Human Immunodeficiency Virus Infection. *Journal of Infectious Diseases* Vol.162, No.4, (October 1990), pp. 862-866, ISSN 1537-6613

Herremans, T., Kortbeek, L. & Notermans, D. W. (2010). A review of diagnostic tests for congenital syphilis in newborns. *European journal of clinical microbiology & infectious diseases* Vol.29, No.5, (May 2010), pp. 495-501, ISSN 1435-4373

Horberg, M. A., Ranatunga, D. K., Quesenberry, C. P., Klein, D. B. & Silverberg, M. J. (2010). Syphilis Epidemiology and Clinical Outcomes in HIV-Infected and HIV-Uninfected Patients in Kaiser Permanente Northern California. *Sexually Transmitted Diseases* Vol.37, No.1, (January 2010), pp. 53-58 ISSN 0148-5717

Johnson, P. D., Graves, S. R., Stewart, L., Warren, R., Dwyer, B. & Lucas, C. R. (1991). Specific syphilis serological tests may become negative in HIV infection. *Aids* Vol.5, No.4, (April 1991), pp. 419-423, ISSN 0269-9370

Kim, D. K., Lee, M. G. & Lee, J. B. (1989). Changes of serum IgG antibody reactivity to protein antigens of Treponema pallidum in syphilis patients after treatment. *Journal of Korean Medical Science* Vol.4, No.2, (June 1989), pp. 63-69, ISSN 1598-6357

Kofoed, K., Gerstoft, J., Mathiesen, L. R. & Benfield, T. (2006). Syphilis and Human Immunodeficiency Virus (HIV)-1 Coinfection: Influence on CD4 T-Cell Count, HIV-1 Viral Load, and Treatment Response. *Sexually Transmitted Diseases* Vol.33, No.3, (March 2006), pp. 143-148, ISSN 0148-5717

Lasfargue, M., Thummler, S., Perelman, S. & de Ricaud, D. (2009). Congenital syphilis: a case report. *Archives de Pédiatrie* Vol.16 No.Suppl 2, (October 2009), pp. S123-126, ISSN 1769-664X

Luger, A., Schmidt, B. & Spendlingwimmer, I. (1977). Quantitative evaluation of the FTA-ABS-IgM and VDRL test in treated and untreated syphilis. *The British Journal of Venereal Diseases* Vol.53, No.5, (October 1977), pp. 287-291, ISSN 0007-134X

Malone, J. L., Wallace, M. R., Hendrick, B. B., LaRocco, A., Tonon, E., Brodine, S. K., Bowler, W. A., Lavin, B. S., Hawkins, R. E. & Oldfield, E. C. (1995). Syphilis and neurosyphilis in a human immunodeficiency virus type-1 seropositive population: Evidence for frequent serologic relapse after therapy. *The American Journal of Medicine* Vol.99, No.1, (July 1995), pp. 55-63, ISSN 1555-7162

Manavi, K. & McMillan, A. (2007). The outcome of treatment of early latent syphilis and syphilis with undetermined duration in HIV-infected and HIV-uninfected patients. *International Journal of Std & Aids* Vol.18, No.12, (December 2007), pp. 814-818, ISSN 0956-4624

Marangoni, A., Moroni, A., Tridapalli, E., Capretti, M. G., Farneti, G., Faldella, G., D'Antuono, A. & Cevenini, R. (2008). Antenatal syphilis serology in pregnant women and follow-up of their infants in northern Italy. *Clinical Microbiology Infection* Vol.14, No.11, (November 2008), pp. 1065-1068, ISSN 1469-0691

Marra, C. M., Boutin, P., McArthur, J. C., Hurwitz, S., Simpson, P. A., Haslett, J. A., van der Horst, C., Nevin, T. & Hook, E. W., 3rd (2000). A pilot study evaluating ceftriaxone and penicillin G as treatment agents for neurosyphilis in human immunodeficiency virus-infected individuals. *Clinical Infectious Diseases* Vol.30, No.3, (March 2000), pp. 540-544, ISSN 1058-4838

Marra, C. M., Longstreth, W. T., Jr., Maxwell, C. L. & Lukehart, S. A. (1996). Resolution of serum and cerebrospinal fluid abnormalities after treatment of neurosyphilis. Influence of concomitant human immunodeficiency virus infection. *Sexually Transmitted Diseases* Vol.23, No.3, (May 1996), pp. 184-189, ISSN 0148-5717

Marra, C. M., Maxwell, C. L., Smith, S. L., Lukehart, S. A., Rompalo, A. M., Eaton, M., Stoner, B. P., Augenbraun, M., Barker, D. E., Corbett, J. J., Zajackowski, M., Raines, C., Nerad, J., Kee, R. & Barnett, S. H. (2004a). Cerebrospinal fluid abnormalities in patients with syphilis: association with clinical and laboratory features. *The Journal of Infectious Diseases* Vol.189, No.3, (February 2004), pp. 369-376, ISSN 0022-1899

Marra, C. M., Maxwell, C. L., Tantalo, L., Eaton, M., Rompalo, A. M., Raines, C., Stoner, B. P., Corbett, J. J., Augenbraun, M., Zajackowski, M., Kee, R. & Lukehart, S. A. (2004b). Normalization of cerebrospinal fluid abnormalities after neurosyphilis therapy: does HIV status matter? *Clinical Infectious Diseases* Vol.38, No.7, (April 2004), pp. 1001-1006, ISSN 1537-6591

Marra, C. M., Maxwell, C. L., Tantalo, L. C., Sahi, S. K. & Lukehart, S. A. (2008). Normalization of serum rapid plasma reagin titer predicts normalization of cerebrospinal fluid and clinical abnormalities after treatment of neurosyphilis. *Clinical Infectious Diseases* Vol.47, No.7, (October 2008), pp. 893-899, ISSN 1537-6591

Marra, C. M., Tantalo, L. C., Maxwell, C. L., Dougherty, K. & Wood, B. (2004c). Alternative cerebrospinal fluid tests to diagnose neurosyphilis in HIV-infected individuals. *Neurology* Vol.63, No.1, (July 2004), pp. 85-88, ISSN 1526-632X

Marra, C. M., Tantalo, L. C., Sahi, S. K., Maxwell, C. L. & Lukehart, S. A. (2010). CXCL13 as a cerebrospinal fluid marker for neurosyphilis in HIV-infected patients with syphilis. *Sexually Transmitted Diseases* Vol.37, No.5, (May 2010), pp. 283-287, ISSN 1537-4521

McMillan, A. & Young, H. (2008). Reactivity in the Venereal Diseases Research Laboratory test and the Mercia(R) IgM enzyme immunoassay after treatment of early syphilis. *International Journal of Std & Aids* Vol.19, No.10, (October 2008), pp. 689-693, ISSN 0956-4624

Palmer, H. M., Higgins, S. P., Herring, A. J. & Kingston, M. A. (2003). Use of PCR in the diagnosis of early syphilis in the United Kingdom. *Sexually Transmitted Infectious* Vol.79, No.6, (December 2003), pp. 479-483, ISSN 1368-4973

Romanowski, B., Forsey, E., Prasad, E., Lukehart, S., Tam, M. & Hook, E. W., 3rd (1987). Detection of Treponema pallidum by a fluorescent monoclonal antibody test. *Sexually Transmitted Diseases* Vol.14, No.3, (July 1987), pp. 156-159, ISSN 0148-5717

Romanowski, B., Sutherland, R., Fick, G. H., Mooney, D. & Love, E. J. (1991). Serologic response to treatment of infectious syphilis. *Annals Internal Medicine* Vol.114, No.12, (June 1991), pp. 1005-1009, ISSN 0003-4819

Rotty, J., Anderson, D., Garcia, M., Diaz, J., Van de Waarsenburg, S., Howard, T., Dennison, A., Lewin, S. R., Elliott, J. H. & Hoy, J. (2010). Preliminary assessment of Treponema pallidum-specific IgM antibody detection and a new rapid point-of-care assay for the diagnosis of syphilis in human immunodeficiency virus-1-infected patients. *International Journal of Std & Aids* Vol.21, No.11, (November 2010), pp. 758-764, ISSN 1758-1052

Schmidt, B. L., Luger, A., Duschet, P., Seifert, W. & Gschnait, F. (1994). Specific IgM tests in syphilis diagnosis. *Hautarzt* Vol.45, No.10, (October 1994), pp. 685-689, ISSN 0017-8470

Schroeter, A. L., Lucas, J. B., Price, E. V. & Falcone, V. H. (1972). Treatment for early syphilis and reactivity of serologic tests. *Jama* Vol.221, No.5, (July 31), pp. 471-476, ISSN 0098-7484

Sheffield, J. S., Sanchez, P. J., Wendel, G. D., Fong, D. W. I., Margraf, L. R., Zeray, F., McIntire, D. D. & Rogers, B. B. (2002). Placental histopathology of congenital syphilis. *Obstetrics and Gynecology* Vol.100, No.1, (July 2002), pp. 126-133, ISSN 0029-7844

Singh, A. E. & Romanowski, B. (1999). Syphilis: Review with emphasis on clinical, epidemiologic, and some biologic features. *Clinical Microbiology Reviews* Vol.12, No.2, (April 1999), pp. 187-209, ISSN 0893-8512

Talwar, S., Tutakne, M. A. & Tiwari, V. D. (1992). VDRL titres in early syphilis before and after treatment. *Genitourinary Medicine* Vol.68, No.2, (April 1992), pp. 120-122, ISSN 0266-4348

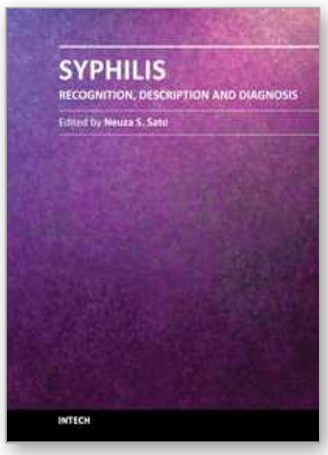
Telzak, E. E., Greenberg, M. S., Harrison, J., Stoneburner, R. L. & Schultz, S. (1991). Syphilis treatment response in HIV-infected individuals. *Aids* Vol.5, No.5, (May 1991), pp. 591-595, ISSN 0269-9370

Walter, T., Lebouche, B., Miailhes, P., Cotte, L., Roure, C., Schlienger, I. & Trepo, C. (2006). Symptomatic Relapse of Neurologic Syphilis after Benzathine Penicillin G Therapy for Primary or Secondary Syphilis in HIV-Infected Patients. *Clinical Infectious Diseases* Vol.43, No.6, (September 2006), pp. 787-790, ISSN 1537-6591

Wicher, V. & Wicher, K. (2001). Pathogenesis of maternal-fetal syphilis revisited. *Clinical Infectious Diseases* Vol.33, No.3, (August 2001), pp. 354-363, ISSN 1058-4838

Workowski, K. A. & Berman, S. (2010). Sexually Transmitted Diseases Guidelines, 2010. *Morbity and Mortality Weekly report* Vol.59, No.RR12, (December 2010), pp. 26-40, ISSN 1545-8601

Yinnon, A. M., Coury-Doniger, P., Polito, R. & Reichman, R. C. (1996). Serologic Response to Treatment of Syphilis in Patients With HIV Infection. *Archives Internal Medicine* Vol.156, No.3, (February 1996), pp. 321-325, ISSN 0003-9926



Syphilis - Recognition, Description and Diagnosis

Edited by Dr. Neuza Satomi Sato

ISBN 978-953-307-554-9
Hard cover, 130 pages
Publisher InTech
Published online 21, November, 2011
Published in print edition November, 2011

Syphilis, a sexually transmitted disease was first described in 15th century, is caused by Treponema pallidum subsp. pallidum and occurs worldwide. This book is a collection of chapters presenting the novel knowledge about the T. pallidum and some historical and up to date information about venereal disease and syphilis. The collection of articles includes: immunological aspects recognition of T. pallidum by the pattern recognition receptors of the innate immune; the whole genome analysis of treponemes and new targets for its molecular diagnosis; some historical aspects of venereal diseases treatment; natural history of syphilis including clinical manifestation and epidemiology; a clinical aspects dealing with psychiatric manifestations of neurosyphilis; spatial and temporal patterns of primary syphilis and secondary syphilis described by the spatial and space-time scan statistics; a commonly used methods for laboratorial diagnosis, the serological response to treatment of syphilis and safety in blood transfusion. I hope this book will be useful for students and research fellows as well for the wide audience.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Neuza Satomi Sato (2011). Serologic Response to Treatment in Syphilis, Syphilis - Recognition, Description and Diagnosis, Dr. Neuza Satomi Sato (Ed.), ISBN: 978-953-307-554-9, InTech, Available from: http://www.intechopen.com/books/syphilis-recognition-description-and-diagnosis/serologic-response-to-treatment-in-syphilis

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen