Screening for Syphilis With the Treponemal Immunoassay: Analysis of Discordant Serology Results and Implications for Clinical Management

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(See the editorial commentary by Hoover and Radolf, on pages 1295–6.)

Background. Screening for syphilis with treponemal chemiluminescence immunoassays (CIA) identifies patients with discordant serology who are not identified with traditional screening methods (eg, CIA-positive, rapid plasma regain (RPR)-negative). We sought to describe the clinical characteristics and management of patients with discordant syphilis serology.

Methods. From August 2007–October 2007, patients with CIA-positive, RPR-negative serology were tested with the Treponema pallidum particle agglutination assay (TP-PA) at Kaiser Permanente Northern California. Clinical and demographic characteristics, prior syphilis history and CIA index values were compared for CIA-positive, RPR-negative patients according to TP-PA status.

Results. Of 21 623 assays, 439 (2%) were CIA-positive and 255/439 (58%) were RPR-negative; subsequently, 184 (72%) were TP-PA-positive and 71 (28%) were TP-PA–negative. TP-PA–positive patients were more likely to be male, HIV-positive, homosexual, previously treated for syphilis (57% versus 9%), with higher median CIA index values (9.8 versus 1.6) (all \(P\) < .0001). After repeat testing, 7/31 (23%) CIA-positive, RPR-negative, TP-PA–negative patients seroreverted to CIA-negative.

Conclusions. TP-PA results in conjunction with clinical/behavioral assessment helped guide the management of patients with CIA-positive, RPR-negative serology. TP-PA-positive patients were both highly likely to have prior syphilis and major epidemiologic risk factors for syphilis. CIA-positive, RPR-negative, TP-PA-negative serology may represent a false-positive CIA in low-prevalence populations.

Identification and control of syphilis through screening, treatment and partner services has been a public health priority since the 1950s in order to prevent the consequences of untreated infection such as pregnancy loss, congenital malformations, and the more recent associations between syphilis and increased risk of HIV acquisition and transmission [1–3]. The past decade has witnessed a resurgence in syphilis in the United States, with an estimated 13 997 cases of primary and secondary syphilis in 2009, many of which occurred in men who have sex with men (MSM), who were diagnosed when they received care in the private sector [4, 5]. Currently, most states in the US require universal screening of pregnant women for syphilis, and screening of high-risk groups such as MSM, in both public and private settings, is recommended at least annually or more frequently according to risk [6].
Syphilis screening in the US has traditionally involved a non-specific, nontreponemal anti-cardiolipin serological test (e.g., rapid plasma reagin [RPR] or Venereal Disease Research Laboratory [VDRL]) with subsequent confirmation of positive results by a specific treponemal test (e.g., Treponema pallidum particle agglutination [TP-PA] or the fluorescent treponemal antibody-absorption test [FTA-ABS]). However, in both North America and Europe, the testing paradigm is shifting towards increased use of treponemal-specific enzyme immunoassays (EIA) and chemiluminescence immunoassays (CIA) for initial syphilis screening, followed by a non-treponemal test (RPR/VDRL) on specimens with positive results [7–11]. The treponemal EIA was initially approved for blood bank screening in the US during the 1980s, and was approved for clinical diagnostic use by the Food and Drug Administration in 2001. In a biennial laboratory survey conducted in California, the volume of treponemal EIA/CIA tests increased from 0 in 2001 to over 390,000 performed in 2007, with a decrease in total RPRs and VDRLs performed from approximately 2.9 to 1.9 million [12]. Increased use of EIA/CIA tests may be attributable to the fact that treponemal immunoassays are highly automated, which improves efficiency and saves costs by eliminating the need for manual pipetting by laboratory personnel. Furthermore, the most recent generation of EIA/CIA tests appears to be more sensitive (95–99%) and specific (98–99%) than prior generations of these assays [13–16]. Given test performance characteristics and improved laboratory efficiency, treponemal EIA/CIA-based screening appears cost-effective from a laboratory standpoint when compared with traditional nontreponemal RPR/VDRL-based screening [17].

Several notable clinical issues have emerged with increasing use of the treponemal EIA/CIA as the initial screening test, including identification of previously unrecognized patients with discordant treponemal and nontreponemal test results (e.g., CIA-positive, RPR-negative). Patients with CIA-positive, RPR-negative results can present diagnostic and treatment challenges for clinicians, especially when these results occur in certain populations such as pregnant women living in areas where syphilis prevalence among reproductive-age women is low.

Although identification of patients with positive CIA/EIA and negative RPR/VDRL serological results will inevitably increase as screening with treponemal immunoassays becomes more widely adopted, there are few clinical data and no national guidelines available for the management of patients with discordant syphilis serological results. Therefore, the objective for this study was to compare differences in demographic, clinical and behavioral characteristics and subsequent clinical management between patients with discordant syphilis serology and a second positive treponemal test (CIA-positive, RPR-negative, TP-PA-positive), and patients with discordant serology and a second negative treponemal test (CIA-positive, RPR-negative, TP-PA-negative).

**METHODS**

We conducted a cross-sectional analysis of patients with syphilis serological test results from Kaiser Permanente Northern California (KPNC), a large integrated health care system which provides care (pharmacy, outpatient medical, and hospital services) to approximately 3.2 million members [18]. This study was approved by the KPNC Institutional Review Board with a waiver of informed consent for study patients.

In August 2007, the Kaiser Permanente Northern California Regional Laboratory replaced the RPR (BD, Franklin Lakes, NJ) with a treponemal CIA (LIAISON, DiaSorin Inc., Stillwater, MN) as the initial test for syphilis screening and diagnostic testing. The LIAISON CIA is a 1-step sandwich assay which produces a light signal according to the quantity of antigen-antibody conjugate detected in a particular specimen. The relative light units emitted are compared with control specimens and a signal-to-cutoff ratio or index value is then reported. Index values range from 0–70 and are interpreted as follows: less than 1.0 is negative, and greater than or equal to 1.0 is positive. Per laboratory protocol, patients with CIA index values less than 1.0 were reported to clinicians as “negative”; these individuals are not included in this analysis.

Patients with positive CIA serology were subsequently reflexively tested with the RPR. Patients with CIA-positive, RPR-negative serology were reflexively tested with the Treponema pallidum particle agglutination assay (TP-PA) (Fujirebio Inc., Malvern, PA). Prior to adopting the CIA, the TP-PA was routinely utilized at KPNC for confirmation of positive RPR tests. Because the performance of the CIA in the KPNC setting was not yet well established, the laboratory continued to use the TP-PA as a referee test in cases of CIA-positive, RPR-negative serology. All serological testing was performed on the same specimen and the results of all three tests were reported simultaneously to providers.

We queried the KPNC Regional Laboratory electronic database for all consecutive patients who were tested with the CIA from 1 August 2007 to 31 October 2007. Patients 18 years and older who were CIA-positive and RPR-negative during the study period were eligible for inclusion in the analysis. For all eligible patients, we obtained CIA optical density cutoff index (ODI) values from the KPNC Regional Laboratory, as well as demographic, clinical and behavioral data from the KPNC electronic health record using a standardized abstraction protocol. Data elements collected included: age, race, sex, sexual orientation, medical history (including HIV status), current pregnancy, and prior history of treated syphilis. Based on the visit type and ordering provider type, we characterized the clinical setting in which testing was conducted. The reason for syphilis testing, stage of syphilis (if diagnosed), treatment and subsequent clinical management were recorded. The reason for testing was determined by reviewing clinician records from the visit when the syphilis serology was ordered. Only patients with
clinician documentation of genital ulcers, rash, neurologic symptoms, or sexual contacts to syphilis were classified under “diagnostic” testing. Finally, we queried KPNC laboratory databases and recorded all historical RPR titers, as well as any follow-up syphilis serology results (repeat CIA, RPR, and/or TPPA) in the 12 months following the initial CIA testing.

The following criteria were used to determine whether patients had a prior history of treated syphilis: 1) documentation in the health record, 2) patient self-report (documented by provider in the clinical encounter note), or 3) prior positive RPR with positive TP-PA or FTA-ABS documented in the KPNC laboratory database prior to August 2007, with subsequent clinical follow-up at KPNC. For patients for whom none of these were documented, a KPNC physician (J. Schapiro) provided names and dates of birth to public health surveillance staff to search state syphilis registries for positive serology prior to August 2007. Results of the registry search were provided to KPNC physicians for further clinical management, if indicated. Patients with no documentation in the KPNC health record and no record in the syphilis serological registry were considered to have no prior history of syphilis.

The $\chi^2$ test was used to compare proportions unless Fisher’s exact test was indicated due to small counts. The Student $t$ test was used to compare the mean and the Wilcoxon test was used to compare non-normally distributed continuous variables (eg, CIA ODI values). A $P$ value of $<.05$ was considered to be statistically significant. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

Results of the 21,623 immunoassays run between August 2007 through October 2007 and subsequent testing performed are illustrated in Figure 1. Overall, 439 (2%) CIA serology tests were positive. After duplicate tests and tests from infants were excluded, 255 unique patients with CIA-positive, RPR-negative serology underwent reflex testing with TP-PA, which resulted in 184 (72%) TP-PA–positive and 71 (28%) TP-PA–negative patients. The demographic characteristics of these patients are described in Table 1.

When compared with TP-PA–negative patients, TP-PA–positive patients were older, more likely to be male, MSM, and HIV-positive (all $P < .001$). TP-PA–positive patients were also more likely to have a prior history of syphilis (57% vs 9%, $P < .0001$). Among patients for whom race was documented ($n = 141/255, 55$%), TP-PA–positive patients were more likely to be African-American than TP-PA–negative patients ($P = .002$). To analyze whether patients with a prior history of syphilis were primarily responsible for the differences in demographic/clinical factors observed, we repeated our analyses after excluding all patients with a documented history of syphilis ($n = 111$), which represented 44% of our study population. CIA-positive, RPR-negative, TP-PA–positive patients were still more likely to be male, MSM, HIV-positive, and were also more likely to be African-American (all $P < .01$) (data not shown). Clinical management, including antibiotic treatment by TP-PA status, is shown in Figure 1. Among patients with no prior history of syphilis, TP-PA–positive patients were more likely to receive antibiotic therapy for the discordant serology result than TP-PA-negative patients (64% versus 10%, $P < .0001$).

Substantial differences were evident in the distribution of CIA ODI values between TP-PA–positive patients and TP-PA–negative patients (Figure 2). TP-PA–positive patients had higher median ODI values (9.8 vs 1.6, $P < .0001$). The majority (65%) of TP-PA–negative patients had ODI values less than 2.0 and 92% had ODI values less than 5, with a range of 1.03–12.00. In contrast, only 9% of TP-PA–positive patients had ODI values less than 2.0, and 30% had ODI values less than 5, with a range of 1.02–70.00. Of the 78/255 (30%) patients with an ODI value greater than 12.00, 100% were also TP-PA–positive.

Of the 255 patients with discordant serology, 100 (39%) were HIV-infected; a large majority of the HIV-infected patients (86%) were CIA-positive, RPR-negative, and TP-PA–positive. Eight patients had CIA-positive, RPR-negative, TP-PA–positive serology and no documentation of prior syphilis, no prior syphilis serology in the past 12 months, and met criteria for possible new late latent disease or latent disease of unknown duration. Only 2 of these 8 patients received antibiotic treatment per the medical record. None of these patients demonstrated neurological symptoms at baseline, none received lumbar puncture, and none manifested neurosyphilis based on follow-up data from the clinical chart.

A total of 37 patients had a prior positive RPR at KPNC in the 12 months prior to the initial CIA test, and were receiving follow-up serological tests following the syphilis diagnosis. Thirty-three (89%) of these patients were CIA–positive, RPR–negative, and TP–PA–positive. Four patients (11%) were TP–PA–negative; these four were also HIV–positive.

Among 28 pregnant women in our study population, 12 (43%) were CIA–positive, RPR–negative, TP–PA–positive and 5 (17%) had a prior history of syphilis. Of the remaining 16 women (57%) who were CIA–positive, RPR–negative, and TP–PA–negative, none had a prior history of syphilis. TP–PA–positive pregnant women were significantly more likely to receive antibiotic therapy for syphilis than TP–PA–negative women (11/12 [92%] vs 2/16 [13%], $P < .0001$).

The clinical setting and reason for syphilis screening or diagnostic testing are detailed in Table 2. The majority of patients had no signs/symptoms (> 60%) and were classified as having received a screening test. TP–PA–positive patients were more likely to have presented with a rash which prompted diagnostic testing ($P = .02$). However, if all potential reasons for diagnostic testing were considered together (ie, genital ulcer, rash, neurologic symptoms, or contact to syphilis), there was no difference...
in the proportion of TP-PA-positive and TP-PA-negative patients receiving diagnostic testing (18% vs 20%).

Results of repeat serological testing are outlined in Figure 1. In summary, 43% of the study population (n = 78 TP-PA-positive and n = 31 TP-PA-negative patients) received repeat serological testing with CIA, RPR and TP-PA within 12 months. Among 78 patients who were initially TP-PA-positive, 0 seroreverted to CIA-negative and 10 (13%) seroconverted to RPR-positive after follow-up testing. The percentage of patients that seroconverted to RPR-positive was unchanged after stratifying for initial antibiotic treatment. None of the seroconverters had signs/symptoms of early syphilis, neurosyphilis or were known to be sexual contacts to early syphilis.

Among 31 patients who were initially TP-PA-negative, 6 (23%) seroconverted to CIA-negative and 1 (3%) seroconverted to CIA-positive, RPR-positive, TP-PA-positive. All seroreversions occurred in patients who were not treated for their initial discordant serological result. The lone seroconversion occurred in a 26-year-old HIV-positive, asymptomatic male with a low CIA ODI value (1.21), who became CIA-positive, RPR-positive (titer 1:4) and TP-PA-positive after repeat testing 6 months later. He had not been treated for his initial discordant result, had no prior history of syphilis, and multiple RPR tests in the 2 years prior to the study period had been negative.

**DISCUSSION**

To our knowledge, this is the first published study to illustrate key differences in demographic, clinical, behavioral, and laboratory characteristics of patients with CIA-positive, RPR-negative serology in a general screening population with the use of treponemal CIA-based screening. The proportion of patients with CIA-positive serology in this health care setting (2%) was lower than positivity rates reported by New York and Chicago laboratories utilizing a variety of treponemal EIAs (6%–14.5%) [10, 19], but similar to the proportion reported for the greater Toronto area of Canada and also by Kaiser Permanente Southern California (2%) using an older generation EIA (TrepChek, Phoenix Biotech, Mississauga, Ontario, CA) [20, 21].
Despite a low prevalence of positive CIA results during the study period, the expected numbers of patients that will be identified in this healthcare setting with reactive syphilis serology under CIA-based screening algorithms will more than double from approximately 600 cases per year with the prior RPR-based algorithms to over 1600 cases per year. Even after exclusion of patients with prior treated syphilis (CIA+/RPR−, 40%), the total number of patients requiring clinical management under CIA-based screening (either CIA-positive/RPR-positive or CIA-positive/RPR-negative without prior syphilis) will nearly double in this low-prevalence population. Despite lower laboratory costs with EIA/CIA-based testing, recent US cost analyses by Owusu-Edusei et al. have demonstrated slightly increased costs per case of syphilis detected with EIA-based algorithms compared with traditional RPR/VDRL-based strategies ($1671 vs $1621). [22] The investigators also predicted a 3-fold increase in the number of follow-ups and a large numbers of patients receiving overtreatment.

Although the optimal management of patients with discordant serology (CIA-positive, RPR-negative) is unclear, our data indicate that in low prevalence settings, the result of a second treponemal test (eg, TP-PA) is useful for clinical management. Recent analyses of treponemal EIA/CIA screening from the Centers for Disease Control and Prevention (CDC) demonstrated that 40% of discordant specimens in low-prevalence settings were subsequently FTA-ABS− or TP-PA−negative; performance of a second treponemal test is currently recommended by the CDC for all EIA/CIA-positive, RPR-negative specimens [19]. CIA-positive, RPR-negative, and TP-PA−positive patients were more likely to have risk factors for syphilis than those who tested TP-PA−negative, a pattern which persisted after excluding patients with a known history of syphilis. In accordance with recommendations by the CDC, at KPNC, patients who are CIA-positive, RPR-negative and TP-PA−positive are assessed and examined for syphilis, staged and offered antibiotic treatment if not previously treated [10]. However, overtreatment may occur for several reasons: patients may not recall previously being diagnosed and treated, resolution of infection may have occurred without treatment, or patients may have previously received antibiotics with activity against Treponema pallidum for unrelated conditions.

The issue of overdiagnosis and overtreatment is especially salient among patients who are CIA-positive, RPR-negative, and TP-PA−negative. Among patients in this category who were retested, 7/31 (23%) seroreverted to CIA-negative without antibiotic treatment, implying that initial CIA results were falsely positive. Woznicova et al. studied 57 patients with EIA-positive and Treponema pallidum hemagglutination assay (TP-HA)-negative serology and tested specimens with multiple serological assays, including Western blot and RPR. After repeated testing, only
5 patients remained EIA-positive and TP-HA–negative; all other serological assays were negative and no patients were determined clinically to have syphilis, albeit the sample size was small [13]. In our population, a HIV-positive patient with CIA-positive, RPR-negative, and TP-PA–negative results later seroconverted to RPR-positive and TP-PA–positive, implying that the CIA may have been more sensitive in detecting early or incubating syphilis than the RPR or TP-PA. Though treponemal tests such as TP-PA do appear more sensitive in primary syphilis than non-treponemal tests [23], there are no published data demonstrating improved sensitivity of treponemal EIA/CIA tests compared with TP-PA and FTA-ABS in early syphilis.

Management of CIA-positive, RPR-negative individuals who are HIV-infected and have not previously been treated for syphilis presents further challenges. Those without prior serology in the past 12 months would be diagnosed with late latent or latent syphilis of unknown duration. Until recently, national guidelines have recommended routine lumbar puncture for HIV-infected patients with late latent syphilis. [6, 24] These recommendations preceded widespread use of the treponemal EIA/CIA, and are based on positive RPR/VDRL findings which reflect disease activity. Decreases in serum RPR titers after treatment reflect similar decreases in VDRL titers in the cerebrospinal fluid, [25] and neurosyphilis is highly unlikely in patients with a negative serum VDRL, [26] and in HIV-infected patients with a serum RPR of less than 1:32. [27] In our study, none of the HIV-infected, CIA-positive, RPR-negative individuals with possible late latent syphilis were evaluated or treated for neurosyphilis, only 2 of 8 were treated for latent syphilis, and none developed neurosyphilis during clinical follow-up. However, larger studies of this population with systematic clinical follow-up are needed.

Optimal management of pregnant women with CIA-positive, RPR-negative serology is another area that warrants further study. Accurate diagnosis of syphilis is especially relevant in this population, as benzathine penicillin is the only recommended treatment for syphilis in pregnancy, but self-reported penicillin allergy is very common among women (11%) [28]; therefore, avoidance of unnecessary treatment during pregnancy is prudent. Most CIA-positive, RPR-negative, TP-PA–negative pregnant women in our study were presumed to have false-positive CIA results and were not treated with antibiotics. Studies of perinatal outcomes in both treated and untreated women with CIA-positive, RPR-negative serology are planned, and will be crucial in creating guidelines for the use of treponemal EIA/CIA in prenatal screening.

Finally, our study raises questions regarding the potential interpretation and utility of the CIA ODI value. Currently, CIA ODI values are reported as positive or negative; our data imply that the quantitative index value may provide useful information, given the significantly higher median and higher range of index values observed in TP-PA–positive individuals.

Figure 2. CIA Cutoff Index Values for CIA+, RPR-patients according to TP-PA status (n = 255). Abbreviations: CIA, chemoluminescence immunoassay; RPR, rapid plasma regain test; TP-PA, Treponema pallidum particle agglutination assay; “+,” positive; “−,” negative.
Notably, all individuals with ODI values $\geq 12.0$ were subsequently TP-PA–positive, implying that TP-PA testing might not be necessary in individuals with high ODI values. A recent study by Wong et al also demonstrated similar findings using a treponemal EIA (Trep-Sure, Phoenix Biotech, Mississauga, Ontario, CAN). [29] Because the range of ODI values varies among the various treponemal immunoassays, index cutoff values that correlate with TP-PA positivity will need to be validated separately for individual assays. Furthermore, the clinical significance of the observed differences in ODI values is still unknown and warrants further study before using these values in a clinical diagnostic setting.

Limitations of our study include potential misclassification of patients with a prior history of syphilis, due to inadequate documentation in the medical record or in state public health registries. Patients who had syphilis outside of the state or whose case was not reported to the syphilis registry would not be captured. Therefore, our results may underestimate the true percentage of patients with a prior syphilis history. Though the KPNC population is generally racially, ethnically and economically representative of the northern California population, it does underrepresent the very poor and very wealthy [30]. Finally, repeat testing and clinical follow-up of patients with discordant results was not uniformly performed by providers, and results of follow-up testing and care may be biased towards higher-risk patients.

Despite these limitations, this study indicates that among patients with CIA-positive, RPR-negative serology, performance of a second treponemal test is useful in low-prevalence settings to guide treatment decisions. CIA-positive, RPR-negative, and TP-PA–positive patients were both highly likely to have had prior syphilis, as well as major epidemiologic risk factors for syphilis. Patients with two positive treponemal serology tests (e.g., CIA, TP-PA) may warrant treatment if no prior treatment has been given. Conflicting treponemal test results (e.g., CIA-positive,
TP-PA-negative), especially with low CIA index values, may represent false positives in low prevalence populations, and repeat testing should be considered. In CIA-positive, RPR-negative populations (regardless of TP-PA status) at high-risk for syphilis, repeat testing should be performed to rule out early syphilis.

Diagnostic limitations exist with any syphilis serological test. Clinicians must still collect other relevant information needed to diagnose and stage patients with suspected syphilis, and continue to use non-treponemal serology to monitor response to treatment. Assessing sexual risk, obtaining syphilis serological and clinical history (including syphilis registry searches by local health departments), performing a complete physical exam, and conducting partner testing continues to be of paramount importance in the diagnosis and staging of patients with suspected syphilis.

Notes

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