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Characterization of the classical biological false-positive reaction in the serological test for syphilis in the modern era

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ABSTRACT

To characterize the CBFP reaction in the modern era, we analyzed the results of parallel rapid plasma reagin (RPR) and *Treponema pallidum* particle agglutination (TPPA) tests from a total of 63,765 blood samples obtained at Zhongshan Hospital in the Medical College of Xiamen University from May 2008 to February 2013. Among the 63,765 tested blood samples, 206 (0.32%) had the CBFP reaction. In multivariate analysis, an increased likelihood of the CBFP reaction was associated with female subjects, subjects ≥80 years old, and subjects between 16 and 35 years old ($P < 0.05$). The CBFP reaction occurred in association with 17 categories of disease, including 60 types of diseases, in the 206 subjects. To our knowledge, a number of these diseases had not been previously reported to be associated with the CBFP in the RPR test, including false labor, megaloblastic anemias, aplastic anemias, redundant prepuce, congenital malformation of heart, and salpingitis. Among the 206 patients with the CBFP reaction, 35 patients were subjected to follow-up for five years. 26 out of 35 these patients were at a 1:1 initial RPR titer, 8 out of 35 patients were at a 1:2 initial RPR titer, and 1 out of 35 patients were at a 1:4 initial RPR titer. 30 subjects had their RPR seroreverted. In our opinion, additional CBFP research using a large sample population will contribute to the identification of additional underlying serious disorders that are not related to syphilis. Such results could be useful for the prediction and diagnosis of these diseases.

1. Introduction

Treponema pallidum (*T. pallidum*) can only be cultured in vivo and can't be stained with simple laboratory stains. In the short term, new molecular tests for syphilis are unlikely to replace serologic tests because molecular tests are relatively expensive, require sophisticated equipment, and also have poor sensitivity for blood samples during *T. pallidum* infections [1–3]. Currently, serologic testing is the most widely used laboratory technique to diagnose syphilis and to monitor the disease after treatment. Serologic tests are divided into two categories: nontreponemal and treponemal antibody tests [1]. Syphilis screening in the U.S. and in some Asian countries has traditionally employed a nonspecific, nontreponemal anticardiolipin serological test (e.g., the rapid plasma reagin (RPR) test or the Venereal Disease Research Laboratory test), with subsequent confirmation of positive results using a specific treponemal test (e.g., the *T. pallidum* particle agglutination (TPPA) test or the fluorescent treponemal antibody-absorption test) [4]. However, in both North America and Europe [5,6], the testing protocol is shifting towards increased use of treponemal-specific enzyme immunoassays (EIA) or TPPA for initial syphilis screening. Onsite RPR test for syphilis screening resulted in a 63.9% of correct diagnosis and treatment among antenatal patients in South Africa [7]. Occasional biological false-positive reactions may occur with any of the serological tests for syphilis, including nontreponemal tests and treponemal tests [8]. The classical biological false-positive (CBFP) reaction in the serological test for syphilis, which is an antibody response to cardiolipin that is not due to *T. pallidum* infection, currently causes problems if treponemal antibody tests are recommended for screening. A number of studies have demonstrated that treponemal assays have fewer false positives than nontreponemal assays [9–11]. In general, the CBFP is more likely to be observed in patients with autoimmune disease, HIV infection, pregnancy and intravenous drug abuse [6]. The aim of this study was to

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characterize the CBFP reaction during syphilis screening in a large-scale study at Zhongshan Hospital in the Medical College of Xiamen University.

2. Materials and methods

2.1. Participants

A total of 172,282 subjects, including inpatients, outpatients and physical examination populations, were screened using syphilitic serologic testing at Zhongshan Hospital in the Medical College of Xiamen University from May 2008 to February 2013. Current law concerning the prevention and treatment of infectious diseases in China requires that the HIV/SYPHILIS/HBV/HCV tests are included as part of the routine screening process for blood donors and inpatients to prevent the spread of infectious diseases. After excluding duplicate sera and subjects for whom only RPR or TPPA results were available, 63,765 subjects who were screened for syphilis using both RPR and TPPA were enrolled in this study. Among the study population, 49,998 subjects having negative RPR and TPPA tests were chosen as non-CBFP group. Disease classification was based on the International Classification of Diseases (ICD)-10. This study was approved by the Institutional Ethics Committee of Zhongshan Hospital in the Medical College of Xiamen University and was in compliance with national legislation and the Declaration of Helsinki guidelines.

2.2. CBFP diagnostic criteria

In our study, the diagnosis of the CBFP reaction during syphilis screening was established on the basis of clinical serological tests, with a positive RPR and a negative TPPA test indicating the CBFP, similar to the European guidelines [6]. All serological testing was performed on the same specimen, and the results of the RPR and TPPA tests were reported simultaneously. The chemiluminescence treponemal antibody immunoassay (CIA) was subsequently performed for each sample to reduce the chance of false-negative TPPA reactions. When both the TPPA and CIA tests were all negative, the positive RPR test was considered a CBFP result (Fig. 1).

2.3. Syphilitic serologic tests

Syphilitic serological tests for each sample, including the RPR test (InTec, Xiamen, China) and the TPPA test (Fujirebio, Tokyo, Japan), were performed for each sample according to the manufacturer's instructions or previously reported protocols [12]. The sera samples reactive for RPR were quantified in 2-fold serial dilutions until the end was determined. All RPR testings (initial and repeat) were done in a blinded fashion to eliminate bias. The initial dilution of serum samples for TPPA reactions was 1:80. Each serum sample with reactive TPPA was further tested using an automated CIA test (Boson Biotechnology Co., Ltd., Xiamen, China) to avoid TPPA false-positive results. Three standard serum samples (Beijing Controls & Standards Biotechnology Co., Ltd., China) with concentrations of 400 mIU/mL, 80 mIU/mL, and 24 mIU/mL were used as controls for the RPR, TPPA, and CIA reactions, respectively. Serum samples that produced conflicting or inconclusive results for a particular technique were tested in duplicate, and the new result was considered accurate.

2.4. Statistical analysis

Statistical analysis was conducted using SPSS version 17.0 for Windows. Chi-square tests were conducted to identify significant differences across groups; $P < 0.05$ (two-sided) was considered of significant difference. We estimated odds ratios with 95% confidence intervals (CIs) using bivariate analysis, and factors for which $P < 0.05$ were further examined using multivariate analysis. Model development was conducted with the inclusion of all selected variables using a step-down approach.

3. Results

3.1. Incidence of the CBFP reaction during syphilis screening

A total of 63,765 subjects were tested for syphilis using both the RPR and TPPA tests at Zhongshan Hospital in the Medical College of Xiamen University from June 2008 to May 2013. In the study subjects, 38,515 subjects were male, and 25,250 subjects were female. Among the

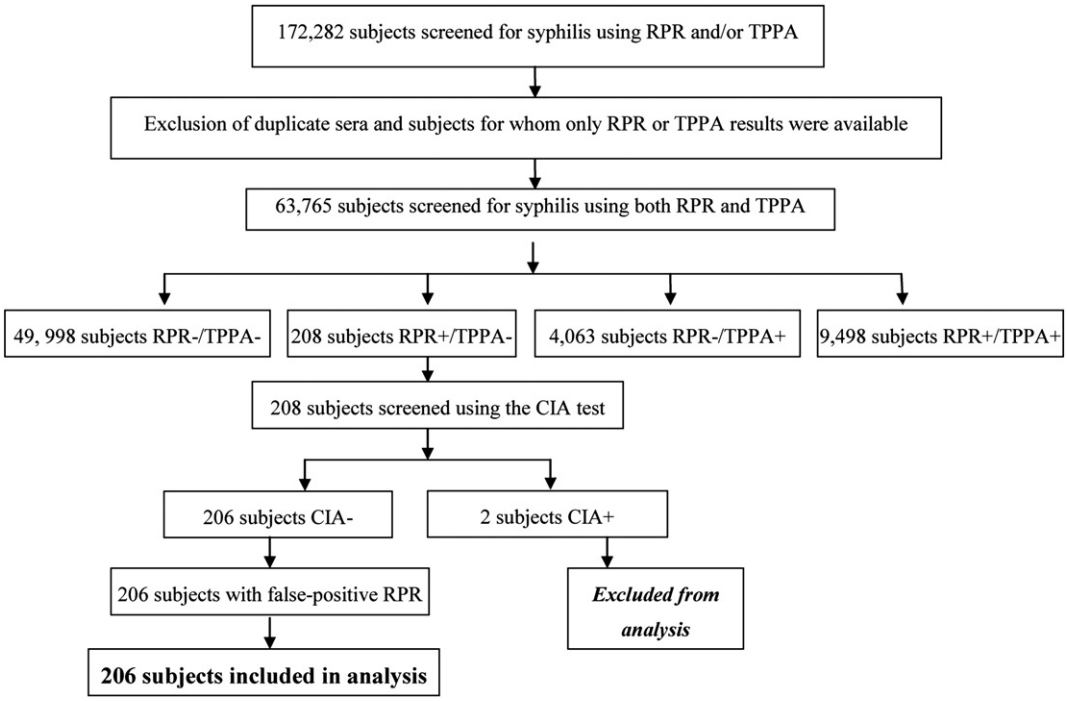

Fig. 1. Selection criteria for subjects included in this study. RPR: rapid plasma reagin; TPPA: *T. pallidum* particle agglutination; CIA: chemiluminescence immunoassay.