International Journal of Infectious Diseases 43 (2016) 51–57

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

A novel ELISA using a recombinant outer membrane protein, rTp0663, as the antigen for serological diagnosis of syphilis☆

Man Xu [a,b,c,1], Yafeng Xie [a,b,c,1], Chuanhao Jiang [a,b,c,1], Yongjian Xiao [a,b,c,d], Xingxing Kuang [a,b,c], Feijun Zhao [a,b,c], Tiebing Zeng [a,b,c], Shuangquan Liu [e], Mingxing Liang [a,b,c], Li Li [a,b,c], Chuan Wang [a,b,c], Yimou Wu [a,b,c,*]

[a] Institution of Pathogenic Biology, Medical College, University of South China, Hengyang 421001, China
[b] Hunan Province Cooperative Innovation Center for Molecular Target New Drug Study, University of South China, Hengyang, China
[c] Hunan Provincial Key Laboratory for Special Pathogens Prevention and Control, University of South China, Hengyang, China
[d] Department of Clinical Laboratory, The Second Affiliated Hospital of University of South China, Hengyang, China
[e] Department of Clinical Laboratory, The First Affiliated Hospital of University of South China, Hengyang, China

ARTICLE INFO

Article history:
Received 23 November 2015
Received in revised form 15 December 2015
Accepted 21 December 2015

Corresponding Editor: Eskild Petersen, Aarhus, Denmark.

Keywords:
Treponema pallidum
Syphilis
Serodiagnosis
Recombinant antigen
Tp0663
ELISA

SUMMARY

Background: The lack of *Treponema pallidum*-specific antigens with highly accurate diagnosis makes the diagnosis of syphilis challenging.
Methods: A soluble recombinant version of a new diagnostic protein Tp0663 has been produced. The serodiagnostic potential of this protein was assessed by screening 3326 serum samples simultaneously evaluated by rapid plasma reagin and *T. pallidum* particle agglutination tests. Kappa (κ) coefficients were used to compare the concordance between clinical diagnosis and the Tp0663-based ELISA or the ARCHITECT Syphilis TP chemiluminescent immunoassay (Abbott GmbH and Co. KG).
Results: Using the results of clinical diagnosis as the gold standard, the sensitivity and specificity of Tp0663 were found to be 98.83% (95% confidence interval (CI) 96.61–99.60%) and 100% (95% CI 99.88–100%), respectively. In comparison, the ARCHITECT Syphilis TP assay was found to have a lower sensitivity (97.27%, 95% CI 94.46–98.67%) and specificity (99.61%, 95% CI 99.32–99.78%). In particular, the ARCHITECT Syphilis TP exhibited a false-positive rate of 0.39%. Moreover, the ELISA was in perfect agreement with the gold standard, with a κ value of 0.99, comparable to that of ARCHITECT Syphilis TP (0.96).
Conclusion: These results identified Tp0663 as a novel serodiagnostic candidate with great potential for developing novel tests for the diagnosis of syphilis.

1. Introduction

Syphilis is a perplexing infection caused by the spirochete *Treponema pallidum*, which is transmitted primarily through sexual contact. It progresses through multiple clinical stages, with typical clinical features including the hard chancre during primary syphilis, followed by a generalized rash and lymphadenopathy during secondary syphilis. Upon resolution of this stage of syphilis, the infection enters an asymptomatic, latent phase lasting from months to decades. Diverse and chronic symptoms emerge during tertiary syphilis, including neurosyphilis, cardiovascular involvement, and gummas.[1]

The laboratory diagnosis of syphilis is dependent on the use of a multitude of serological tests due to the fact that *T. pallidum* cannot be cultured in vitro;[2,3] this is in contrast to most bacterial diseases, for which a definite diagnosis can be made by direct detection of the pathogen. The common serological tests for the diagnosis of syphilis are divided into two categories: non-treponemal tests and treponemal tests. The non-treponemal tests, such as the rapid plasma reagin (RPR) test and the venereal disease research laboratory (VDRL) test, are used mainly to determine serological

☆ This work was performed at the Institution of Pathogenic Biology, Medical College, University of South China; Hunan Province Cooperative Innovation Center for Molecular Target New Drug Study, University of South China; Hunan Provincial Key Laboratory for Special Pathogens Prevention and Control, University of South China, Hengyang, China.

* Corresponding author. Tel.: +86 13707340050; fax: +86 734 8282907.
E-mail address: yimouwu@sina.com (Y. Wu).

[1] Man Xu, Yafeng Xie, and Chuanhao Jiang contributed equally to this work.

http://dx.doi.org/10.1016/j.ijid.2015.12.013

activity and to monitor the therapeutic effect. Despite the advantages of these tests, which are widely available, inexpensive, and simple to perform, the results require further confirmatory screening for the detection of Treponema-specific antibodies. The treponemal tests, such as the *T. pallidum* particle agglutination (TPPA) assay, the *T. pallidum* hemagglutination (TPHA) assay, and the fluorescent treponemal antibody absorption (FTA-ABS) test, are used to detect the specific treponemal antibodies. Nevertheless, these tests are labor-intensive and highly operator-dependent. Furthermore, they exhibit poor sensitivities in the detection of early syphilis.[4]

In recent years, automated enzyme immunoassays (EIAs) and chemiluminescence immunoassays (CIAs) have also been developed for the serodiagnosis of syphilis. These assays, using one or more of the recombinant *T. pallidum* proteins TpN15 (Tp0171), TpN17 (Tp0435), TpN44.5 (TmpA, Tp0768), and TpN47 (Tp0574),[5,6] have gained the interest of researchers in the field of syphilis diagnosis because they are objective, reproducible, automated, and computerized.[7] More importantly, these tests have led some large laboratories in the USA to screen patients with the reverse algorithm, which begins with a treponemal test; reactive tests are followed by a quantitative non-treponemal test and discordant samples must be rescreened with a second and different treponemal test.[8,9] Although these proteins have been used in the serodiagnosis of syphilis, there is no general agreement as to which protein antigens are best in terms of serodiagnostic performance, and surface-exposed proteins may have superior sensitivity to the currently used proteins due to their immediate exposure to the immune system. Therefore, it is of great importance to evaluate new recombinant antigens with highly accurate diagnosis for use in serological testing for syphilis.

The purpose of this study was to analyze the diagnostic potential of recombinant antigen Tp0663, a 28-kDa *T. pallidum* subsp. *pallidum* outer membrane protein that has previously been reported in several works as being reactive with sera from syphilis patients.[10,11] The sensitivity and specificity of the recombinant antigen were evaluated using sera collected from 3326 individuals, and subsequently compared with the recently launched ARCHITECT Syphilis TP test, a CIA that exhibits excellent ability to automate testing in high-throughput instrumentations. The results showed that the *T. pallidum* protein Tp0663-based immunoglobulin G (IgG) ELISA exhibited higher overall sensitivity and specificity than the ARCHITECT Syphilis TP. Therefore, Tp0663 represents a promising new candidate that could potentially be incorporated into automated diagnostic tests for syphilis.

2. Materials and methods

2.1. Genomic DNA of Treponema pallidum subsp. pallidum Nichols strain

The *T. pallidum* subsp. *pallidum* Nichols strain used in this study was a generous gift from Tianci Yang (Zhongshan Hospital, Medical College of Xiamen University, Xiamen, China) and was propagated in adult New Zealand white rabbits, as described elsewhere.[12,13] All animal experiments were approved by the Institutional Review Committee of the University of South China.

2.2. Serum samples

This study was approved by the Human Ethics Committee of the University of South China and was performed in compliance with the Declaration of Helsinki guidelines and national legislation. Informed consent was obtained from all participants. Human serum samples were collected from The First Affiliated Hospital, University of South China (Hengyang, China), The Second Affiliated Hospital, University of South China (Hengyang, China), The First People's Hospital of Changde (Changde, China), and Hunan Provincial People's Hospital (Changsha, China) between February 2015 and May 2015. The serological detection of syphilis in each sample (from 3326 subjects) was performed using the RPR and TPPA tests, which were performed in accordance with the manufacturers' instructions, after duplicate tests were excluded. All serological tests were performed on the same serum sample, and the results of the two tests were reported simultaneously. The subjects in this study included 948 individuals undergoing routine health examinations, 1358 outpatients, and 1020 inpatients. These subjects underwent syphilis detection for screening (if asymptomatic), for diagnosis (if symptomatic), or to monitor the effects of therapy. In accordance with the staging criteria described in the literature,[14] the clinical diagnosis of syphilis was determined by combining serological tests and disease history (including clinical signs and symptoms, and/or the patient's sexual history). Primary syphilis is generally characterized by painless chancre and serous fluids from the lesion, usually coupled with regional lymphadenopathy; there has generally been sexual contact with a person with syphilis, and laboratory confirmation involves dark-field examination and/or positive results of both RPR and TPPA to confirm the diagnosis of syphilis. Secondary syphilis is often characterized by a generalized rash, mucocutaneous lesions, and lymphadenopathy; there is generally a history of sexual exposure to a person with syphilis or primary syphilis, and reactive serological tests (RPR and TPPA) are used to confirm the diagnosis. Latent syphilis is asymptomatic, with a possible history of infection supported by a reactive RPR or TPPA result. An onset of infection within the last 2 years is referred to as early latent syphilis and a duration of latent syphilis >2 years is defined as late latent syphilis. Tertiary syphilis is considered as syphilis presenting with typical clinical symptoms, such as gummatous lesions, neuropsychiatric illness, and cardiovascular involvement, and a history of primary, secondary, or latent syphilis, in addition to clinical confirmation by positive results of both RPR and TPPA. The diagnostic tests used to confirm the serum samples are listed in Table 1.

2.3. Recombinant protein expression and purification

The *tp0663* gene was amplified by PCR using the forward primer 5′-CCGGAATTCATGAAACAGGGCTGTTTTAT 3′ (*Eco*RI) and the reverse primer 5′-CCCAAGCTTTCATTTGCCGCTCTCTCCTT 3′ (*Hin*dIII) and then cloned into pET-28a expression vector. This construct was transformed into *Escherichia coli* BL21 (DE3) cells and the positive clone was confirmed by DNA sequencing. Protein expression was induced overnight at 30 °C with 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). Bacteria were harvested and lysed in a buffer containing 50 mM Tris–HCl (pH 7.8), 300 mM NaCl, 10 mM imidazole, 20% glycerol, and 1% Triton X-100. His-tagged protein was purified by affinity chromatography using Ni-NTA beads (Qiagen, Inc., Hilden, Germany). The concentration of the recombinant protein was estimated using a bicinchoninic acid protein assay kit (Pierce, Rockford, USA).

2.4. Western blotting analysis

Recombinant protein Tp0663 was electrophoresed in a 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel and then transferred to a nitrocellulose membrane (Merck Millipore, Darmstadt, Germany). After blocking at room temperature for 2 h with phosphate-buffered saline (PBS) containing 5% nonfat milk and 0.05% Tween 20 (PBSTM),