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Research Note

Anti-*Treponema pallidum* IgA response as a potential diagnostic marker of syphilis

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ABSTRACT

Objectives: Serological tests for syphilis detect mainly total Ig, IgM or IgG antibodies. We aimed to evaluate the specific IgA response in syphilis patients according to disease stage.
Methods: A serum IgA-enzyme immunoassay was developed using commercially available microplates coated with recombinant treponemal antigens and an anti-IgA-conjugate. To define a cut-off, we used 91 syphilis positive and 136 negative sera previously defined by the rapid plasma reagin and the *Treponema pallidum* particle agglutination results. Then we determined the intra- and inter-assay precisions, diagnostic sensitivity according to the clinical stage (in 66, 55 and 42 sera from primary, secondary and latent syphilis patients, respectively) and specificity (in 211 sera from people with conditions different to syphilis). IgA values were further measured in 71 sera from patients with previously treated syphilis.
Results: The newly developed IgA-enzyme immunoassay showed a good discrimination between negative and positive samples with intra- and inter-assay variation coefficients <20%. The sensitivity was 80.3% (95% CI, 70.0–90.6), 100.0% (95% CI, 99.1–100.0) and 95.2% (95% CI, 87.6–100.0) in primary, secondary and latent syphilis, respectively, and the specificity was 98.1% (95% CI, 96.0–100.0). Further, IgA values were negative in 61.3% (38/62) of patients with previously treated syphilis.
Discussion: Our findings suggest serum IgA as a sensitive and specific marker of syphilis and its detection could be used as a screening assay for active infection. Further evaluation is needed in prospective longitudinal field studies. **Islay Rodríguez, Clin Microbiol Infect 2023;▪:1**

Introduction

Syphilis is a sexually transmitted disease caused by *Treponema pallidum* subsp. *pallidum*. Its serological diagnosis is done by combining non-treponemal and treponemal assays. The sensitivity and specificity of tests vary according to the assay, antigen nature and clinical stage of the disease. Treponemal tests are specific and detect Ig subclasses such as IgG, IgM or IgG/IgM or total Ig including IgG, IgA and IgM. Nontreponemal tests detect antibodies against cardiolipin and lecithin and are useful for the detection of active infection and for monitoring treatment response [1–3].

IgA is present both in serum and secretions of mucosal surfaces [4,5]. Its specific detection is considered a high-quality serological marker for other infectious diseases and it only persists as long as antigenic stimulation exists [6,7]. Except for one recent report [8], IgA has not been systematically evaluated in syphilitic patients for more than 25 years, despite being a potentially good marker for active infections, whose sensitivity and specificity could equal or exceed that of existing tests. Previous studies were done using native *T. pallidum* antigens obtained from intratesticular inoculation of rabbits [9,10]. The availability of recombinant treponemal polypeptides during the last decades has facilitated the development of new specific treponemal tests.

Aiming to investigate new serological markers of syphilis, an exploratory study for the detection of serum IgA using recombinant treponemal antigens in a new enzyme immunoassay (EIA) was performed in sera from patients with different clinical and serological profiles.

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Methods

Samples

Samples were collected in Swiss and Cuban institutions, previously tested by rapid plasma reagin (RPR), *Treponema pallidum* particle agglutination (TPPA) or *Treponema pallidum* hemagglutination (TPHA), IgM-EIA and Liaison XL Ig screening test. PCR in ulcer swabs was done as previously described [11].

The first development set of sera consisted of 91 RPR and TPPA/TPHA positive and 136 RPR and TPPA/TPHA negative samples. Positive sera (31 with low RPR titers [≤4] and 60 with high RPR titers [≥8]) were from syphilis patients at any disease stage.

A second set of samples was used to evaluate the new test. For sensitivity, 66, 55, and 42 sera from patients with primary, secondary and latent syphilis, respectively, were tested and for specificity, 211 sera from people without syphilis. Further, 62 sera from people with a history of previously treated syphilis based on serological results (time-point of infection unknown) were analysed.

Development and evaluation of IgA-EIA

Briefly, SYPH IgM microplates (Dia.Pro) coated with synthetic recombinant treponemal proteins (p47, p17 and TmpA) were employed. Sera were always added 1/101 diluted and mixed with neutralizing reagent (anti-IgG) in the plate. Results were reported as optic density (OD) values. First, six positive and two negative sera, defined by RPR and TPPA, were tested with 1/5 000, 1/10 000, 1/20 000 and 1/40 000 dilutions of anti-IgA conjugated horseradish peroxidase (Dia.Pro). The 1/20 000 dilution of anti-IgA conjugate was selected due to better discrimination between positive and negative results with less saturation for highly positive samples.

The IgA-EIA with the 1/20 000 conjugate dilution was then applied to the first set of samples. The cut-off OD value for assessing a sample as positive or negative was defined as the mean OD of all negative samples plus two standard deviations. Intra- and inter-assay coefficients of variation were also determined using five replicas of one negative and one positive serum tested in the same plate and replicates of them tested on five different days, respectively.

Sensitivity and specificity were evaluated with the second set of samples with the determined cut-off.

Statistics

Results were expressed with descriptive statistics measures. Sensitivity and specificity of tests were calculated using Epidat version 4.2 software (Galicia Council, Spain).

Ethics

This study was approved by the ethics committees of the Tropical Medicine Institute "Pedro Kourí", Havana, Cuba (reference number CEI-IPK 23-20) and Zurich, Switzerland (reference number 2016-01518).

Results

The median OD of the positive samples was 20.2 times higher than that of the negative samples (1.948 [interquartile range 0.724–2.802] versus 0.097 [interquartile range 0.049–0.178]; $p \leq 0.0001$) in the development set of sera (Fig. 1a). With the determined cut-off OD of 0.4, 6/136 negative and 15/91 positive samples were attributed to the wrong category. Of the 15 false-negative samples, 8 had a low RPR titer (≤4) and 7 had a high RPR titer (≥8). The intra- and inter-assay coefficients of variation were 6.8% and 6.5% for the positive sample and 12.7% and 17.0% for the negative sample, respectively.

In the evaluation set, the IgA OD values were high in secondary and latent syphilis sera and lower in primary syphilis (Fig. 1b). The

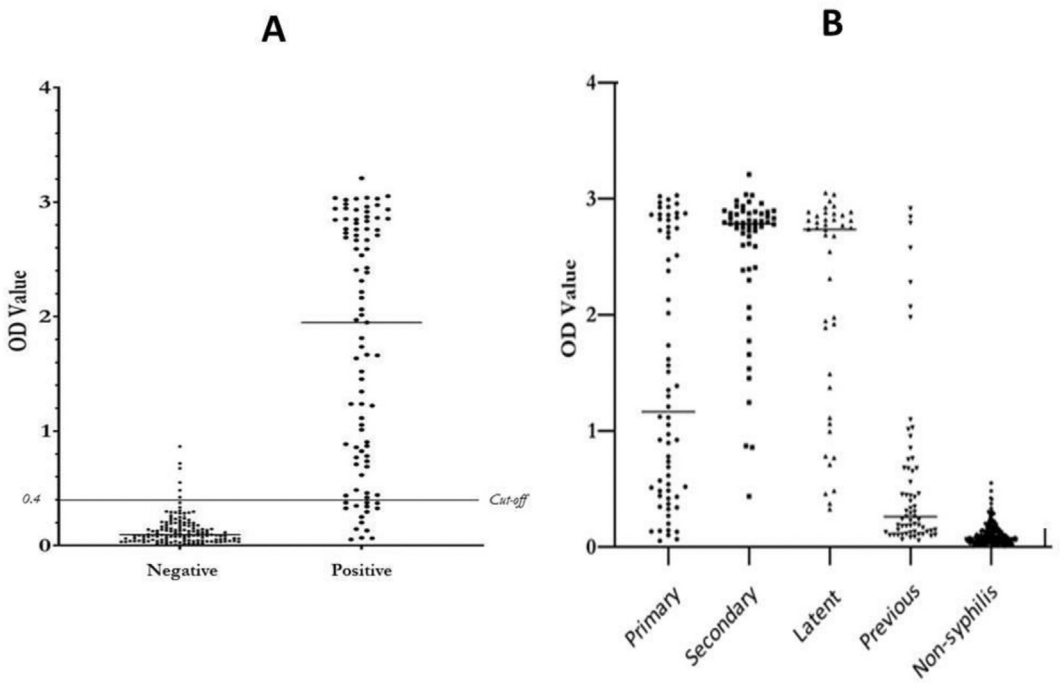

Fig. 1. Anti-*Treponema pallidum* IgA-enzyme immunoassay results. (a) Comparing syphilis positive and negative sera using the selected conjugate dilution. (b) In sera from patients with active primary, secondary, or latent syphilis, patients with residual antibodies of previous syphilis and nonsyphilis patients.

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Table 1
Sensitivity and specificity of IgA-enzyme immunoassay. Sensitivity is shown according to clinical stage of syphilis and RPR titers

Clinical stage	Sensitivity (95% CI)	Sensitivity according to RPR titer		Specificity (95% CI)
		RPR titer	Sensitivity (95% CI)	
Primary syphilis (n = 66)	80.3% (70.0–90.6)	≤4 (n = 28)	60.7% (40.8–80.6)	—
		≥8 (n = 38)	94.7% (86.3–100.0)	—
Secondary syphilis (n = 55)	100.0% (99.1–100.0)	≤4 (n = 2)	100% (75.0–100.0)	—
		≥8 (n = 53)	100% (99.1–100.0)	—
Latent syphilis (n = 42)	95.2% (87.6–100.0)	≤4 (n = 8)	87.5% (58.3–100.0)	—
		≥8 (n = 34)	97.0% (89.9–100.0)	—
Previously treated syphilis (n = 62)	38.7% (25.8–51.6)	—	—	—
Nonsyphilis samples (n = 211[a])	—	—	—	98.1% (96.0–100.0)

RPR, rapid plasma regain.
[a] Including 73 potentially cross-reactive sera (47 with Epstein-Barr virus IgM, 3 with cytomegalovirus IgM, 8 with rubella virus IgM, 12 with *Chlamydophila pneumoniae* IgA, and 3 of *Toxoplasma gondii* primo infections), 10 false-positive sera reacting with one syphilis test alone (rapid plasma reagin, *Treponema pallidum* particle agglutination, or Liaison XL) and 128 syphilis negative sera (47 from laboratory routine without further specification and 81 from blood donors).

overall sensitivity of IgA-EIA was 90.8% (95% CI; 86.0–95.5). It was lowest in primary syphilis, especially in those cases with low RPR titer, and highest in secondary syphilis (Table 1). IgA was also positive in 38.7% of previously treated syphilis cases.

In primary syphilis patients with positive PCR on chancre exudates (n = 22), IgA was negative in 45.5% (10/22). These patients were sampled 1 to 21 days (median 5.5 days) after the onset of the lesion. The remaining 12 patients with positive IgA had chancres for 3 to 60 days (median 14 days). Likewise, 22.7% (5/22), 36.4% (8/22) and 31.8% (7/22) of the PCR-positive patients had negative RPR, TPPA, and IgM-EIA.

The overall specificity was 98.1% (95% CI, 96.0–100.0). Only three EBV IgM-positive samples and one blood donor sample were false positive (Table 1, Fig. 1b).

Discussion

The new IgA-EIA showed good discrimination between negative and positive samples, with acceptable intra- and inter-assay coefficient of variation, supporting its robustness. The sensitivity was good to excellent depending on the clinical stage of syphilis.

In primary syphilis, all serological tests missed 22.7 to 45.5% of cases that were only detected by PCR. This reaffirms the necessity of combining serology and molecular testing in patients with ulcer lesions [12,13]. Therefore, screening tests with superior sensitivities are needed [14].

The highest sensitivities of IgA-EIA were found in samples from patients with secondary and latent syphilis, stages that correspond to the hematogenous spread of the pathogen.

The specificity of the IgA assay was excellent, which is in contrast to Julian et al. [9], who reported IgA in 20% of nonsyphilitic individuals who had never been infected with *T. pallidum*. Their high cross-reaction percentage could be due to the antigen mixture extracted from rabbit testicular syphilomas [9].

Our findings are comparable to those from Pham et al. [8], who developed an in-house immunochromatographic IgA test using a mixture of chimeric recombinant antigens [8]. Their sensitivity and specificity were 96.1% to 100% and 84.7% to 99.4%, respectively. They suggested the potential utility of this assay as an accurate confirmatory test when combined with a treponemal total antibody screening test. However, when we analysed patients with residual antibodies of previously treated syphilis, IgA antibodies were cleared in over 60%. We thus conclude that IgA might not be an optimal confirmatory marker in general because confirmatory tests need to be positive also in patients with past infection.

This study has limitations; i) reference comparator test were not identical in the three participating laboratories which may influence the robustness of the evaluation, ii) A relatively small number of samples was tested. Although the results are very promising with no inferiority compared to RPR and TPHA/TPPA, more studies are needed to confirm the utility of the IgA test.

In conclusion, our results demonstrate that serum IgA constitutes a specific response to *T. pallidum* infection and could be useful as a serological marker of an active infection. The IgA-EIA test demonstrates high sensitivity and excellent specificity and it can be automated. Thus, it might be used as a screening assay for active infection. Further evaluation is needed in larger prospective longitudinal studies.

Author contributions

IR and RL conceptualized the project, and all authors designed the study. IR, PPB and RL were responsible for data collection, and all authors analysed and interpreted the data. IR drafted the manuscript, and all authors were involved in critically revising the manuscript for important intellectual content. All authors approved the final manuscript and accepted final responsibility for the decision to submit for publication.

Transparency declaration

The authors declare that they have no conflicts of interest.

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References

[1] Peeling RW, Mabey D, Kamb ML, Chen XS, Radolf JD, Benzaken AS. Syphilis Nat Rev Dis Primers 2017;3:17073. https://doi.org/10.1038/nrdp.2017.73.
[2] Ghanem KG, Ram S, Rice PA. The modern epidemic of syphilis. N Engl J Med 2020;382(9):845–54. https://doi.org/10.1056/NEJMra1901593.
[3] Bosshard PP. Usefulness of IgM-specific enzyme immunoassays for serodiagnosis of syphilis: comparative evaluation of three different assays. J Infect 2013;67(1):35–42. https://doi.org/10.1016/j.jinf.2013.03.011.
[4] Woof JM, Kerr MA. The function of immunoglobulin A in immunity. J Pathol 2006;208:270–82. https://doi.org/10.1002/path.1877.
[5] De Sousa Pereira P, Woof JM. IgA: structure, function, and developability. Antibodies 2019;8(4):57. https://doi.org/10.3390/anti8040057.
[6] De Decker S, Vray M, Sistek V, Labeau B, Enfissi A, Rousset D, et al. Evaluation of the diagnostic accuracy of a new dengue IgA capture assay (Platelia Dengue IgA Capture, Bio-Rad) for dengue infection detection. PLos Negl Trop Dis 2015;9(3):e0003596. https://doi.org/10.1371/journal.pntd.0003596.
[7] Fresse AS, Sueur JM, Hamdad F. Diagnosis and follow-up of genital chlamydial infection by direct methods and by detection of serum IgG, IgA and secretory IgA. Indian J Med Microbiol 2010;28(4):326–31. https://doi.org/10.4103/0255-0857.71823.

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[8] Pham MD, Wisec A, Garcia ML, Van H, Zheng S, Mohamed Y, et al. Improving the coverage and accuracy of syphilis testing: the development of novel rapid, point-of-care test for confirmatory testing of active syphilis infection and its early evaluation in China and South Africa. E Clin Med 2020;24:100440. https://doi.org/10.1016/j.eclinm.2020.100440.

[9] Julian AJ, Logan LC, Norins LC, Cohen IR. Immunoglobulin class of natural human antibodies reactive with Treponema pallidum. Infect Immunol 1970;1(6):555–8. https://doi.org/10.1128/iai.1.6.555-558.1970.

[10] Schmitz JL, Gertis KS, Mauney C, Stamm LV, Folds JD. Laboratory diagnosis of congenital syphilis by immunoglobulin M (IgM) and IgA immunoblotting. Clin Diag Lab Immunol 1994;1(1):32–7. https://doi.org/10.1128/cdli.1.1.32-37.1994.

[11] Gayet-Ageron A, Laurent F, Schrenzel J, Charton B, Jimenez-Getaz G, Tangomo M, et al. Performance of the 47-kilodalton membrane protein versus DNA polymerase I genes for detection of *Treponema pallidum* by PCR in ulcers. J Clin Microbiol 2015;53:976–80. https://doi.org/10.1128/JCM.03444-14.

[12] Noda AA, Rodríguez I, Grillova L, Bosshard PP, Lienhard R. Accuracy of PCR and serological testing for the diagnosis of primary syphilis: both tests are necessary. Int J STD AIDS 2019;30(11):1087–94. https://doi.org/10.1177/0956462419859764.

[13] Glatz M, Juricevic N, Altwegg M, Bruisten S, Komericki P, Lautenschlager S, et al. A multicenter prospective trial to asses a new real-time polymerase chain reaction for detection of Treponema pallidum, herpes simplex-1/2 and Haemophilus ducreyi in genital, anal and oropharyngeal ulcers. Clin Microbiol Infect 2014;20(12):O1020–7. https://doi.org/10.1111/1469-0691.12710.

[14] Park IU, Fakile YF, Chow JM, Gustafson KJ, Jost H, Schapiro JM, et al. Performance of treponemal tests for the diagnosis of syphilis. Clin Infect Dis 2019;68(6):913–8. https://doi.org/10.1093/cid/ciy558.

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