Differential influence of race and environment on indeterminate reactivities to non-treponemal and treponemal antigens by immuno-chromatographic dual syphilis rapid test

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Key words: Syphilis, immuno-chromatographic rapid test, false positivity, race, environment, Central Africa, France

Received: 27/06/2018 - Accepted: 27/05/2019 - Published: 06/06/2019

Abstract

Introduction: syphilis rapid test results may be influenced by numerous environmental and genetic factors. Methods: the proportion of false positive syphilis non-treponemal (NT) and treponemal (T) test results using immuno-chromatographic dual syphilis rapid test on serum from Cameroonian blacks (n=103) versus French blacks (n=104) or French caucasians (n=51), all HIV-negative and free of clinical syphilis, was examined. Results: Black individuals in Cameroon had a significantly higher frequency of false positive NT or T tests than black individuals in France. Black individuals in France had a higher frequency of indeterminate NT tests as compared to caucasians in France. Conclusion: both racial and environmental factors may affect immuno-chromatographic dual syphilis rapid testing.

The Pan African Medical Journal. 2019;33:90. doi:10.11604/pamj.2019.33.90.16437

This article is available online at: http://www.panafrican-med-journal.com/content/article/33/90/full/

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Introduction

Syphilis rapid test results may be influenced by numerous environmental and genetic factors [1-6]. We herein report on the frequency of false-positive non-treponemal (NT) and treponemal (T) tests using a dual syphilis rapid test on serum from black individuals living in Central Africa versus black and caucasian individuals living in France.

Methods

Collection of serum aliquots (conserved at -20°C) from routine serological testing according to medical prescription of black adults in Cameroon, and black and caucasian adults in France, as controls, was used, in agreement with institutional ethical and research review boards of Laboratoires Litto Labo, Hygiene Mobile in Cameroon [7, 8] and Assistance Publique de Paris (processing number 1922081) in France. All selected individuals had given their oral informed consent for sampling, were HIV-negative and free of any clinical manifestations of syphilis. No personal identifiers were collected.

All sera selected for the study were negative by reference gold standard NT and T syphilis serology, including RPR test for NT antibodies (Arlington Scientific Inc., Springville, Utah, USA), and ELISA test for T. pallidum-specific IgG+IgM antibodies (DiaSorin, Vercelli, Italy). The rapid point-of-care immuno-chromatographic dual test RDT DPP® Syphilis Screen & Confirm Assay (Chembio Diagnostics Systems Inc., Medford, NY, USA) was used for simultaneous detection of both NT and T antibodies, as described [9, 10]. Readings were made independently by two technicians exactly 15 minutes after the addition of the last running buffer. For one given line, concordant interpretations gave the final results, e.g. negative, positive or doubtful.

Results

A total of 103 sera were prospectively collected in Cameroon, and 155 sera in France, from 104 blacks and 51 caucasians. All sera negative by the reference gold standard for syphilis serology were further tested by the syphilis rapid test. Indeterminate NT reactivities by syphilis rapid test on sera from individuals from Central Africa (23.3%) were more frequent than that observed on sera from black and caucasian individuals living in France (12.5% and 1.9%, respectively) (P<0.05 and P<0.0004, respectively) (Table 1). The prevalences of indeterminate NT reactions were high in black than caucasian people living in France (P<0.04).

Treponemal T reactivities by syphilis rapid test on sera from individuals from Central Africa (9.7%) were more frequent than that observed on sera from black and caucasian individuals living in France (1.9% and 0.0%, respectively) (P<0.02 and P<0.04, respectively), whereas the prevalences of indeterminate T reactivities were similar in black and caucasian people living in France. When considering all NT or T reactivities, sera from individuals from Central Africa were more frequently indeterminate by syphilis rapid test than sera from black and caucasian individuals living in France. Furthermore, black individuals living in France were more frequently indeterminate by dual syphilis rapid test than caucasian individuals living in France.

Finally, when considering double NT and T reactivities, sera from individuals from Central Africa were more frequently false positive by syphilis rapid test than sera from black and caucasian individuals living in France, whereas the prevalences of indeterminate NT and T reactivities were similarly low in black and caucasian people living in France.

Discussion

In the present study, false positive NT or T reactivities as well as indeterminate syphilis results by immuno-chromatographic dual syphilis rapid test in individuals without a clinical history of syphilis and syphilis-negative by reference serology were more frequently observed in black individuals living in Central Africa than in black individuals living in France and in caucasian individuals living in France. Furthermore indeterminate NT reactivities by dual syphilis rapid test were more frequently observed in black individuals living in France than caucasian individuals living in France. The risk of false positive dual syphilis rapid test with positive NT and T bands was significantly higher in black individuals living in Central Africa than in black and caucasian individuals living in France, while black and caucasian individuals living in France showed similarly low risk of false positivity by rapid test. These observations point that both racial and environmental factors may affect the results of the immuno-chromatographic dual syphilis rapid test.
Similar to HIV, false positive syphilis rapid test reactions can occur due to febrile illnesses, immunizations, pregnancy, connective tissue disease and malignancy [11]. In addition, false positive syphilis reactions may also be observed in the context of immune activation occurring during malaria, hepatitis C, Chagas disease, tuberculosis and leprosy [11]. Environmental, hygienic and dietary factors may furthermore contribute to immune activation and polyclonal antibody production [7]. Genetic variability may finally account for differences in the frequency of doubtful test results. Africans have more HLA diversity and class II haplotypes as compared with other ethnic groups including caucasians [4, 6, 7], resulting in varying immunological responses to non-HIV infectious diseases and thus the nature and frequency of cross-reactive antibodies [7, 12, 13].

**Conclusion**

Taken together, our observations emphasize the absolute need for rapid tests to undergo evaluation in the specific environments in which they will be deployed.

**What is known about this topic**

- Syphilis rapid test results may be influenced by numerous environmental and genetic factors.

**What this study adds**

- Both racial and environmental factors may affect immuno-chromatographic dual syphilis rapid testing;
- Our observations emphasize the absolute need for rapid tests to undergo evaluation in the specific environments in which they will be deployed.

**Authors’ contributions**

Francois-Xavier Mbopi-Keou, Ginette Claude Mireille Kalla, Ralph-Sydney Mboumba Bouassa, Fru Angwafo III, Laurent Belec: conceived, designed and performed the experiments. Esther Voundi Voundi, Frédéric Talla, Ralph-Sydney Mboumba Bouassa, Fru Angwafo III: analyzed the data. Francois-Xavier Mbopi-Keou, Ralph-Sydney Mboumba Bouassa: contributed to reagents/materials/analysis tools. Mohammad-Ali Jenabian, Francois-Xavier Mbopi-Keou, Ginette Claude Mireille Kalla, Ralph-Sydney Mboumba Bouassa, Laurent Belec: wrote the paper. All authors have contributed to the manuscript. All authors have read and agreed to the final manuscript.

**Acknowledgements**

Raw data of the study are available from the Laboratoire Litto-Labo, Douala, Cameroon, and the Laboratoire de Virologie, Hôpital Européen Georges Pompidou, Paris, France.

**Table**

**Table 1**: non-treponemal and treponemal reactivities by the immuno-chromatographic test TDR DPP® Syphilis Screen & Confirm Assay (Chembio Diagnostics Systems Inc., Medford, NY, USA)

**References**

1. World Health Organization. The global elimination of congenital syphilis: rationale and strategy for action. The global elimination of congenital syphilis: rationale and strategy for action. 2007. Accessed 07 January 2017.

2. Hill AV, Allsopp CE, Kwiatkowski D, Taylor TE, Yates SN, Anstey NM et al. Extensive genetic diversity in the HLA class II region of Africans, with a focally predominant allele, DRB1*1304. Proc Nat Acad Sci USA. 1992;89(6):2277-2281. [PubMed] [Google Scholar]
3. Clerici M, Butto S, Lukwiya M, Saresella M, Decli S, Trabattoni D et al. Immune activation in Africa is environmentally-driven and is associated with upregulation of CCR5. Italian-Ugandan AIDS Project. AIDS. 2000 Sep 29;14(14):2083-92. PubMed | Google Scholar

4. Cao K, Moormann AM, Lyke KE, Masaberg C, Sumba OP, Doumbo OK et al. Differentiation between African populations is evidenced by the diversity of alleles and haplotypes of HLA class I loci. Tissue antigens. 2004;63(4):293-325. PubMed | Google Scholar

5. Klarkowski D, O'Brien DP, Shanks L, Singh KP. Causes of false-positive HIV rapid diagnostic test results. Expert review of anti-infective therapy. 2014;12(1):49-62. PubMed | Google Scholar

6. Marks M, Yin YP, Chen XS, Castro A, Causer L, Guy R et al. Meta-analysis of the performance of a combined treponemal and non-treponemal rapid diagnostic test for syphilis and yaws. Clin Infect Dis. 2016;63(5):627-33. Google Scholar

7. Mbopi-Keou FX, Ndjoyi-Mbiguino A, Talla F, Pérez H, Kebe K, Matta M et al. Association of inconclusive sera for human immunodeficiency virus infection with malaria and Epstein-Barr virus infection in Central Africa. J Clin Microbiol. 2014;52(2):660-2. PubMed | Google Scholar

8. Jenabian MA, Costinuki CT, Talla P, Robin L, Tonen Wolye S, Mboumba Bouassa RS et al. Potential for false-positive results with serological assays for HIV in Central Africa: implications for the HIV serodiagnosis algorithm according to the 2015 consolidated WHO Recommendations for resource-constrained countries. AIDS Res Hum Retroviruses. 2017;33(11):1077-1079. PubMed | Google Scholar

9. Castro AR, Esfandiari J, Kumar S, Ashton M, Kikkert SE, Park MM et al. Novel point-of-care test for simultaneous detection of non-treponemal and treponemal antibodies in patients with syphilis. J Clin Microbiol. 2010;48(12):4615-4619. PubMed | Google Scholar

10. Guinard J, Prazuck T, Péré H, Poirier C, LeGoff J, Boedec E et al. Usefulness in clinical practice of a point-of-care rapid test for simultaneous detection of non-treponemal and Treponema pallidum-specific antibodies in patients suffering from documented syphilis. Int J STD AIDS. 2013;24(12):944-50. PubMed | Google Scholar

11. Morshed MG, Singh AE. Recent trends in the serologic diagnosis of syphilis. Clin Vacc Immunol. 2015;22(2):137-147. PubMed | Google Scholar

12. Alves C, Souza T, Meyer I, Toralles MB, Brites C. Immunogenetics and infectious diseases: special reference to the mayor histocompatibility complex. Braz J Infect Dis. 2006;10:122-131. PubMed | Google Scholar

13. Santos T de J, Costa CM, Goubau P, Vandamme AM, Desmyter J, Dooren SV et al. Western blot sera-indeterminate individuals for human T-lymphotropic virus I/II (HTLV-I/II) in Fortaleza (Brazil): a serological and molecular diagnostic and epidemiological approach. Braz J Infect Dis. 2003;7(3):202-209. PubMed | Google Scholar
|                          | Cameroonian black | French black | French caucasian | \( P \) |
|--------------------------|-------------------|--------------|-----------------|--------|
| **n**                    | 103               | 104          | 51              |        |
| **Nontreponemal reactivity (n; %)** |                   |              |                 |        |
| Reference serology, RPR *| 0 (0.0)           | 0 (0.0)      | 0 (0.0)         |        |
| Dual rapid test, nontreponemal line | 24 (23.3) | 13 (12.5) | 1 (1.9) | <0.05  |
| Cameroonian Black versus French Black |                   |              |                 | <0.05  |
| Cameroonian Black versus French Caucasian |                   |              |                 | <0.0004|
| French Black versus French Caucasian |                   |              |                 | <0.04  |
| **Treponemal reactivity (n; %)** |                   |              |                 |        |
| Reference serology, IgG+IgM ELISA b | 0 (0.0)           | 0 (0.0)      | 0 (0.0)         |        |
| Dual rapid test, treponemal line | 10 (9.7)          | 2 (1.9)      | 0 (0.0)         |        |
| Cameroonian Black versus French Black |                   |              |                 | <0.02  |
| Cameroonian Black versus French Caucasian |                   |              |                 | <0.04  |
| French Black versus French Caucasian |                   |              |                 | NS     |
| **Indeterminate syphilis reactivities (n; %)** c | 34 (33.0) | 15 (14.4) | 1 (1.9) |        |
| Cameroonian Black versus French Black |                   |              |                 | <0.002 |
| Cameroonian Black versus French Caucasian |                   |              |                 | <0.05  |
| French Black versus French Caucasian |                   |              |                 | <0.04  |
| **Active syphilis false positivity (n; %)** d | 9 (8.7)           | 2 (1.9)      | 0 (0.0)         |        |
| Cameroonian Black versus French Black |                   |              |                 | <0.04  |
| Cameroonian Black versus French Caucasian |                   |              |                 | <0.04  |
| French Black versus French Caucasian |                   |              |                 | NS     |

* The reference serology for non-treponemal line was the Rapid Plasma Reagin test (Arlington Scientific Inc., Springville, Utah, USA); sera whose RPR titers were ≥ 1:2 were considered as positive;

b The reference serology for the treponemal line was the indirect ELISA for *Treponema pallidum*-specific IgG and IgM antibodies directed to 15-, 17-, and 47-kDa recombinant proteins as antigens (DiaSorin, Vercelli, Italy);

c Statistical analyses were accrued out using Fisher exact test using GraphPad Prims software (version 5, La Jolla, CA, USA);

d Indeterminate syphilis reactivities was defined as one or two bands by the immune-chromatographic dual test TDR DPP® Syphilis Screen & Confirm Assay;

e Active syphilis false positivity was defined as two positive bands by the immune-chromatographic dual test TDR DPP® Syphilis Screen & Confirm Assay.

n: Number; NS: Not significant