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Brief Original Article

Treponema pallidum infection rate in patients attending the general hospital of Benguela, Angola

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Abstract

Introduction: The objectives of this study were to estimate the rate of infection by *Treponema pallidum* and co-infection with Human Immunodeficiency Virus (HIV) in individuals attending the General Hospital of Benguela (GHB), Angola, to verify the Rapid Plasma Reagin (RPR) test performance for its diagnosis when compared with other RPR tests, and to compare a rapid treponemal test with the *Treponema pallidum* hemagglutination assay (TPHA).

Methodology: This is a cross-sectional study carried out between August 2016 and January 2017, at the GHB, 546 individuals attending the emergency room, the outpatient service or hospitalized at the GHB were included.

All the samples were tested at the GHB with the routine hospital RPR test and a rapid treponemal test. The samples were then transported to the Institute of Hygiene and Tropical Medicine (IHMT) where RPR testing and TPHA testing were performed.

Results: The rate of *T. pallidum* active infection, demonstrated by a reactive RPR and TPHA result, was 2.9%, of which 81.2% corresponded to indeterminate latent syphilis and 18.8% to secondary syphilis. HIV co-infection was detected in 62.5% of individuals diagnosed with syphilis. Past infection, defined as a non-reactive RPR and reactive TPHA test, was diagnosed in 4.1% of individuals.

Conclusions: The high rate of syphilis/HIV co-infection emphasizes the urgent requirement for adequate sexually transmitted infections (STIs) screening, prevention and treatment programs. In addition, implementation of quality control measures within RPR testing protocols at GHB are needed, including training for laboratory personnel, adequate equipment and introduction of other rapid testing.

Key words: Syphilis; *Treponema pallidum*; RPR; TPHA; Angola; Africa.

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Introduction

Syphilis is an infection caused by the bacterium *Treponema pallidum* and is primarily sexually transmitted [1]. Since syphilis is a cofactor of Human Immunodeficiency Virus (HIV) infection, its progression may be more rapid in HIV-infected individuals. *T. pallidum* infection is also associated with an increased risk of developing syphilis transmission/acquisition of HIV [2,3].

Syphilis infection is a global concern with 7.1 million people newly infected with *T. pallidum* and 0.2 million related deaths in 2020 [3]. In Africa, the total number of new cases was 3.4 million in 2008 [4]. Existing studies on the prevalence of syphilis in Africa are mainly performed in pregnant women, and estimated to be 3.5 to 6.5% [5].

In Angola, knowledge about infection with *T. pallidum* is very limited, and data relating to its prevalence is scarce and inconsistent, even when generated by credible institutions. Therefore, we decided to perform the present study to further understand this subject and to analyze if the tools used for syphilis diagnosis are correct.

Methodology

In this study, patients were not involved in the definition of study design, outcome measures or patient' recruitment procedures. The study community is located in a rural region of Africa, where health literacy is very low and where the connection with the medical community is distant.

It is a cross-sectional survey, carried out between August 2016 and January 2017, at the General Hospital of Benguela (GHB) in Angola that included subjects with permanent residence in Benguela province, either attending the emergency room, the outpatient service or hospitalized.

The study protocol was approved by the Scientific and Ethics Committee of the GHB. To be included, participants or caregivers (for subjects unable to give

consent) had to sign an informed consent. Translation was available for non-Portuguese speakers. Access to the patient's personal data was limited to the research physician.

The total population who attended the GHB in the homologous period of the previous year was 36732 (N) individuals. The minimum sample size required to estimate prevalence of this infection was calculated based upon data from previous studies conducted in Angola using the formula: $n \geq NZ^2P\ (1 - P)/d^2\ (N - 1) + Z^2P\ (1 - P)$, where n = required sample size; N = number of patients attending GBH in the previous year homologous period; Z = 1.96 (0.975 quantile of a normal distribution for a 95% confidence interval); P = 0.5 (infection prevalence in the Angolan population estimated from previous studies) and d = 0.05 (margin of error). The calculated minimum sample size was 381 subjects, but an additional 165 individuals were included in a total of 546 patients. After defining the sample and the duration of its collection (six months), the number of patients to be included per month was obtained by dividing the sample needed (546) by the total number of months.

One blood sample was collected from each participant by venipuncture and placed into two dry tubes, which were centrifuged in the central laboratory of the GHB. One tube was for performing the rapid plasma reagin (RPR) test (Rapid Labs Ltd, Essex, United Kingdom), used at the GHB as the screening non-treponemal test. Before centrifuging, a blood drop from the syringe was used by the researcher to perform a specific rapid anti-*T. pallidum* antibody detection test (Anti Syphilis Test, Laboquick, İzmir, Turkey) and a HIV rapid test (INFO Turklab Anti-VIH1/2, Izmir, Turkey).

The remaining serum in the second tube was frozen at -20 ºC for six months (according to applicable Good Laboratory Practices), transported to the laboratory of the Instituto de Higiene e Medicina Tropical (IHMT), Lisbon, Portugal, where further tests were performed: non-treponemal RPR (Macro-VueTM RPR Card tests, Maryland, USA), treponemal test *Treponema pallidum* hemaglutination assay (TPHA - Fortress, Belfast, United Kingdom), and HIV rapid tests (Hexagon, Wiesbaden, Germany). In IHMT, an HIV-1/2 Ag/Ab Combo test (Alere, UK) was performed in all blood samples. A western blot GenscreenTM HIV-1/2 Version 2 (Bio-Rad, Marnes-la-Coquette, France) was performed in every sample showing discrepant HIV results between GHB and IHMT.

Infection by *T. pallidum* in this study was determined according to the following criteria:

i) Active infection (if reactive RPR and reactive TPHA): RPR reactive samples if both GHB and IHMT were considered true positives. If RPR result was discrepant between GHB and IHMT, an additional RPR was performed at IHMT, as a tiebreaker.

An active infection could be: a) primary syphilis (reactive RPR and reactive TPHA, with primary ulcer); b) secondary syphilis (reactive RPR and reactive TPHA, with secondary skin lesions); c) indeterminate latent syphilis (reactive RPR and reactive TPHA, without symptoms).

ii) Past infection: (if non-reactive RPR and reactive TPHA).

iii) Absence of infection by *T. pallidum* if non-reactivity was observed in both RPR and TPHA tests.

The seropositivity rate of the *T. pallidum* and HIV infections studied and the sociodemographic factors (gender, age) were evaluated. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) V.26. Seropositivity rate of the studied infections and respective confidence intervals were estimated at 95% (Wilson method).

Ethics approval

The study protocol was approved on 07/11/2014 by the Scientific and Ethics Committee of the General Hospital of Benguela (N/ref: 26/014).

Results

A total of 546 patients enrolled in the study from 1 August 2016 to January 31 2017; 60.8% (332) were female and 39.2% (214) were male; age range was from 0 to 81 years and mean age was 33 years (SD ± 16.9). None of the sociodemographic variables was associated with *T. pallidum* infection at 5% level of significance.

Non-treponemal GHB RPR tests were reactive in 7.1% (39/546, 95% CI 5.09-9.59), while IHMT RPR were reactive in 2.9% (16/546, 95% CI 1.66-4.68). In the case of treponemal tests, 7.1% (39/546, 95% CI 5.09-9.59) of the samples showed reactivity in the rapid test (RT) and 7% (38/546, 95% CI 5.01-9.47) were reactive in TPHA.

The rate of active infection by *T. pallidum* was 2.9%, while past infection was 4.1% (22/546, 95% CI 2.60 to 6.12) (Table 1).

Secondary syphilis was diagnosed in 18.8% (3/16, 95% CI 4.07 to 45.70) participants with active *T. pallidum* infection, and 81.2 % (13/16, 95% CI 54.30 to 95.93) had indeterminate latent syphilis. HIV co-infection was identified in 62.5% (10/16, 95% CI 35.43 to 84.80) and all coinfected individuals had indeterminate latent syphilis, representing 76.9%

(10/13, 95% CI 46.16 to 94.95) of the total indeterminate latent syphilis (coinfected and non-co-infected).

Discussion

In this study, 7% of the participants had antibodies against *T. pallidum* indicating a past or present infection, therefore they were potential transmitters at some point in their lives. The rate of active *T. pallidum* infection was 2.9%, with 2.4% of infected individuals considered as indeterminate latent syphilis because of unawareness of the time of infection.

Available data from low and middle-income countries (LMICs) are based on studies of syphilis and HIV co-infection. The high rate of syphilis and HIV has been documented, with prevalence of HIV/syphilis co-infection in LMIC studies ranging from 0.73% to 69.2% [9]. The fact that transmission mechanisms are the same also increases the probability of co-infection. In the present study, the percentage of HIV infection in individuals with syphilis (syphilis/HIV) was high (62.5%), which is compatible with data presented by Mutagoma *et al*. [6].

The test routinely used for syphilis diagnosis at the GHB is the RPR. There was a high discrepancy (29/546 samples) between results of the RPR tests performed at GHB and at IHMT. This discrepancy could arise from differences in suppliers, storage conditions (temperature and humidity), subjectivity when reading the test and unsuitable laboratory equipment. RPR tests performed at GHB were reactive in 39 participants, among which only 13 also had a reactive RPR test at IHMT and therefore a true result. In the other 26 cases, a second RPR test was performed at IHMT due to discrepancy between results that identified past infection in 20 individuals (non-reactive IHMT, second RPR and reactive TPHA) and 6 cases with no previous infection (RPR and TPHA non-reactive), but with treponemal test negative at GHB. Conversely, three patients were considered false negative (non-reactive GHB RPR test and reactive IHMT RPR and IHMT TPHA). This means that a high number of patients were over treated and three did not receive treatment. However, if the RPR reactive results would be confirmed by a specific test like the TPHA test, the results would change and the end result would be better patient management. There was little discrepancy between the specific point-of-care (POC) *T. pallidum* antibody test and the TPHA test, suggesting that like the TPHA, the POC treponemal test has good sensitivity and specificity. This is the reason why these were recommended by the World Health Organization (WHO) in 2006 for use in low resource countries like Angola [7].

The present study had some limitations. The fact that the inclusion of patients was directly related to attendance at GBH could lead to increased infection prevalence compared to the general population, namely in relation to the rate of syphilis HIV co-infection. Furthermore, there is no gold standard in syphilis diagnostic testing due to the fact that *T. pallidum* antibody tests are not 100% specific and sensitive, and that clinical features are crucial in symptomatic syphilis diagnosis. Serological testing is the only diagnostic tool in latent syphilis. These constraints lead to diagnostic difficulties.

In conclusion, the present study emphasizes the importance of quality control protocol implementation when undertaking RPR or POC screening tests, whenever it is impossible to have good non-POC test performance. It is recommended that POC tests should be used in hospitals. It is important to highlight that this is an urgent requirement that may be a common issue in other LMICs.

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Table 1. Interpretation of treponemal and non-treponemal tests.

Interpretation	RPR*		TPHA	RT**	Sub-total		Total	
	GHB	IHMT			n	(%)	n	(%)
Active infection	R	R	R	R	13***	(2.4)	**16**	**(2.9)**
	NR	R	R	R	3	(0.5)		
Past infection	R	NR	R	R	20	(3.7)	**22**	**(4.1)**
	NR	NR	R	R	2	(0.4)		
No infection	R	NR	NR	R	1	(0.2)	**508**	**(93.0)**
	R	NR	NR	NR	5	(0.9)		
	NR	NR	NR	NR	502	(91.9)		
Total					546	(100.0)	**546**	**(100.0)**

*In case of a discrepant result between the General Hospital of Benguela (GHB) and Instituto de Higiene e Medicina Tropical (IHMT) the rapid plasma reagin (RPR) tests, an additional RPR test was performed at the IHMT as a tiebreaker. **In case of discrepant result between rapid anti-*T. pallidum* antibody detection test (RT) and IHMT TPHA tests, an additional treponemal test *Treponema pallidum* hemaglutination assay (TPHA) was performed at the IHMT as a tiebreaker. ***3/13 with syphilis cutaneous lesions. Reactive-R; Non-Reactive-NR; number – n.

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