DIAGNOSIS OF SECONDARY SYPHILIS THROUGH ORAL LESIONS IN TWO PATIENTS WITH NEGATIVE SEROLOGY: CASE REPORTS

ABSTRACT
Syphilis is a sexually transmitted infection, and oral lesion can be the first manifestation. The serology test, such as Venereal Disease Research Laboratory test, is accepted as an effective testing strategy for detecting syphilis, although false-negative reaction can occur, and oral lesions may be pivotal to achieve the diagnosis. We report two cases of seronegative secondary syphilis, a human immunodeficiency virus positive patient and a no HIV positive patient, whose histopathological exams were pivotal to achieve the diagnosis of syphilis. The serology may be negative in secondary syphilis and the oral lesions may represent the unique method to diagnostic.

Keywords: oral lesion; syphilis; HIV; diagnosis.

INTRODUCTION
Syphilis is a sexually transmitted infection caused by Treponema pallidum. The number of reported cases still increases around the world, and, in Brazil, the National Program for Sexually Transmitted Diseases and AIDS Control estimates an annual incidence of 937,000 new cases of syphilis in the sexually active population. The incubation period for syphilis is 21–30 days after the initial contact with the microorganism and may vary from 10–90 days, depending on the virulence of the parasite, as well as the host response. The disease is classified into early (primary, secondary or latent) and late (also known as tertiary) or early congenital and late congenital syphilis. Oral lesions are mainly associated with secondary syphilis and can be the first clinical manifestation. Nevertheless, due to its clinical heterogeneity, depending on the stage of syphilis, the diagnosis of oral syphilis lesions can be a challenge to the clinicians. A nontreponemal serologic test, such as Venereal Disease Research Laboratory (VDRL) test, is accepted as an effective testing strategy for detecting syphilis, although false-negative reaction can occur, particularly in HIV positive individuals, delayed diagnosis or misdiagnosis of syphilis occurs frequently. Sometimes, false-negative specific antitreponemal antibodies, such as Fluorescent Treponemal Antibody Absorption (FTA-ABS), can also occur. In such cases, oral manifestations and their histopathological exam may be pivotal to achieve the diagnosis of syphilis.

The aim of present paper was to report two cases of seronegative secondary syphilis in a HIV positive patient and a no HIV positive patient, who had the diagnosis of syphilis obtained by biopsy of oral lesions.

CASES PRESENTATION
Case 1
A 37-years-old man was referred to the Oral Medicine Service of the Universidade Federal Fluminense with a history of multiple aphthous lesions, which partially resolved in three months. The patient was HIV-positive for four years. The CD4 lymphocytes count was 596 cells/mm³ and the viral load was 5,838 copies/mL at the time of the first oral evaluation appointment. The patient was not under any medication and a previous VDRL test (performed two weeks before) was negative. The patient had never had any major opportunistic infections since his first HIV positive test.

Oral examination revealed painful smooth ulcerations with slightly raised borders and granular center, as well as erythematous patches, on the buccal mucosa, tongue dorsal surface and soft palate.
Diagnosis of secondary syphilis through oral lesions

The clinical diagnosis was deep mycosis or syphilis. The patient signed the informed consent and an incisional biopsy of the buccal mucosa and tongue was performed.

Histopathological examination revealed hyperplasia, parakeratosis and papillomatosis of the epithelium and mononuclear and polymorphic inflammatory cells exocytosis. Microabscesses were also present. The lamina propria showed a dense and diffuse chronic inflammatory infiltrate composed mainly by plasma cells. The inflammatory infiltrate extended to the deeper area of the lamina propria and also showed a perivascular pattern. Obliterative endarteritis characterized by endothelial swelling was also observed. The Warthin Starry stain showed the presence of spirochetal organisms (Figure 2). Neither spores nor hyphae of Candida spp were identified in Periodic Acid Schiff (PAS) stain and the cytopathological analysis was negative for candidiasis.

Another VDRL exam was requested, which was again negative. Based on the clinicopathological findings and despite a negative VDRL, the final diagnosis was syphilis. The infectologist initiated a penicillin treatment. A FTA-ABS test and a third VDRL were requested, which were positives. The VDRL presented at titer 1:128. One week after the beginning of the treatment, the oral lesions had completely resolved (Figure 1 G,H) and, after two months, the VDRL at titer 1:16.

**Case 2**

A 29-years-old woman was referred to the Oral Medicine Service for evaluation of pain and migratory oral lesions with two month of duration. Her medical history revealed that she presented hepatitis B in 2009. Extraoral exam was normal, and oral exam revealed erythematous patches on the labial and buccal mucosa (Figure 3A-D). The patient presented previous VDRL, FTA-ABS and HIV tests (performed one week before) negatives. The clinical diagnosis was deep mycosis or geographic stomatitis. The patient signed the informed consent, and an incisional biopsy of the buccal mucosa was performed and a new VDRL requested.

**Figure 1 –** Clinical aspects of oral syphilis before and after treatment. Ulcers with slightly raised borders and granular center, as well as erythematous patches, on the tongue dorsal (A-C), tongue ventral surface (D), buccal mucosa (E), and soft palate (F). One week after the beginning of the treatment, the oral lesions had completely resolved (G,H).

**Figure 2 –** Histopathological aspects of oral syphilis. Fragment showed hyperplasia, parakeratosis and papillomatosis of the epithelium and mononuclear and polymorphic inflammatory cells exocytosis (A-B). The lamina propria showed a dense chronic inflammatory infiltrate composed mainly by lymphocytes and plasma cells with a perivascular pattern (C). The Warthin Starry stain showed the presence of spirochetes (D).
Histopathological exam revealed buccal mucosa specimen depicting mild architecture changes on the left side and inflammatory changes on the rest of the tissue. Features observed were epithelial hyperplasia, parakeratosis, papillomatosis and neutrophils exocytosis with microabscess. The connective tissue demonstrated superficial and perivascular plasma cell inflammatory infiltrate, and the blood vessels exhibited swelling endothelial cells (Figure 4).

Neither spores nor hyphae of Candida spp were identified in PAS stain, and the cytopathological analysis was negative for candidiasis.

The histopathological exam suggested syphilis, and the VDRL was positive (titer 1:128). The patient was referred to the Infection Diseases Clinic and initiated a penicillin treatment. After of the treatment, the oral lesions had completely resolved (Figure 3E-H).

DISCUSSION

The classical dilemma of the diagnosis of secondary syphilis faced by many clinicians occurs due to the variability of the lesions\(^\text{1,3,11}\). Skin manifestations of secondary syphilis occur in 75% of patients, and the primary chancre is still present in 15% of these patients\(^\text{11}\). Various oral manifestations can be of diagnostic importance and are present in one-third to one-half of patients\(^\text{12}\).

The diagnosis of secondary stage can be performed by specific and non-specific serological tests\(^\text{8,11,12}\). Non-specific tests, such as VDRL, are the most common diagnostic tests used to diagnose syphilis and can be useful for screening large numbers of patients\(^\text{8,12}\). VDRL becomes positive in 4 to 8 weeks after acquiring the infection and the sensitivity approaches 100% in secondary syphilis due to the high antibody titers\(^\text{8}\). VDRL is an inexpensive and useful screening test and is reactive in most patients with secondary and latent disease\(^\text{8}\). However, in 1–2% of patients false-negative VDRL can occur due to prozone phenomenon. This occurs due to an inappropriate ratio of antibody versus antigen preventing their agglutination\(^\text{8,9}\). This prozone phenomenon is frequently found in pregnancy and HIV infection. The incidence of prozone phenomenon is very low in non-HIV patients with syphilis, ranging from 0 to 0.4%\(^\text{9}\). Beyond VDRL, which is a non-specific test, other specific tests are used for screening\(^\text{9}\). Generally, FTA-ABS can be considered a very sensitive test in all stages of syphilis, which is still considered the golden standard\(^\text{8}\). Very rare cases (0.35%) of false-negative FTA-ABS can occur and can be found in HIV infection, autoimmune diseases and pregnancy.

In the case 1, patient had two negative VDRL and in the case 2, VDRL and FTA-ABS were negative. The patient 1 was HIV positive,

![Figure 3](image3.png)

**Figure 3** – Clinical aspects of oral syphilis before and after treatment. Erythematous mucosal plaque with mild white ulcerated center on the lip (A), buccal (B), palate (C) mucosa and the tongue (D). E-H pictures demonstrate partial regression of the lesions after seven days of treatment.

![Figure 4](image4.png)

**Figure 4** – Histopathological aspects of oral syphilis. Histopathological aspects exhibiting epithelial hyperplasia, parakeratosis, papillomatosis and neutrophils exocytosis (A). On the right side, in a higher magnification, epithelium with microabscess (B). The connective tissue demonstrated an intense chronic plasma cell inflammatory infiltrate on the surface and in depth perivascular (C) and the increased blood vessels with edematous endothelial cells (D).
which justifies the presence of false-negative results, although they are rare. False-negative results occur because of impaired response of B lymphocytes to Treponema pallidum, or due to high antibody titers. However, patient 2 was not HIV-positive neither pregnant, showing that the phenomenon can occur in both tests and in any patient. In the literature, we did not find any case of false-negative in patient with hepatitis B. To our knowledge, this is the first case reported in the literature.

The occurrence of prozone phenomenon may be decreased when laboratories perform appropriate testing and dilutions. This is performed by diluting the patient’s serum to bring the antibody concentration into the zone of equivalence. Nevertheless, many hospital laboratories do not routinely test for the prozone phenomenon and, therefore, a laboratory error must also be considered in such cases of false-negative results. In the cases presented in this paper, the exams were performed in different references laboratories(13). In such cases, oral manifestations and their histopathological exam may be pivotal to achieve the diagnosis of syphilis.(7,10). However, histopathological features are variable and the diagnosis of syphilis may also represent a challenge for pathologists.(7,10).

In these present cases, there were no skin lesions, but the patients presented oral manifestations of syphilis, which allowed the diagnosis. Several clinical differences have been described in many case reports of patients with HIV co-infection(15). Oral lesions at the secondary stage persist from few days up to eight weeks and have a variety of clinical appearance, which may lead to a misdiagnosis.(15). Usually, oral lesions present as multiple painful mucous patches, ulcers, deep ulcers and are located in the soft palate, dorsum of the tongue and vestibular mucosa.(5,1,3).

In these cases, the patients were diagnosed with secondary syphilis through the biopsy of oral lesions, which presented different aspects, including erythematous patches and ulcers on the buccal mucosa, tongue and palate. Moreover, oral lesions and their histopathological exam may represent the unique method to diagnostic.(4,5,10,11).

The histopathological characteristics of secondary syphilis are as variable as the clinical manifestations(10). Whereas the changes are often non-specific, findings of proliferation and obliterating endothelial, perivascular infiltrates with a preponderance of plasma cells, and epithelium psoriasiform hyperplasia support the diagnosis of syphilis.(3,7,10). Similar histopathological features with the remarkable presence of hyperplasia, papillomatosis and microabscess in the epithelium were observed in these cases. The lamina propria showed a dense and diffuse chronic inflammatory infiltrate composed mainly by lymphocytes and plasma cells. In addition, silver stain and dark-field microscopy are useful to identify spirochetes in tissue sections and are helpful to achieve the diagnosis(15). In this patient, Warthin Starry stain showed the presence of spirochetal organisms, confirming the diagnosis of syphilis. Based on the histopathological findings, another VDRL and FTA-ABS were requested, which were positive.

CONCLUSION

In conclusion, the serology may be negative in secondary syphilis in HIV patients and no HIV patients, making diagnosis difficult. In these cases, the oral lesions and histopathological exam may represent the unique method to diagnostic.

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