Usefulness of Immunohistochemial Staining With Antitrepenomal Antibodies in the Diagnosis of Syphilis☆

Utilidad de la inmunohistoquímica con anticuerpos antitreponema en el diagnóstico de la sífilis

To the Editor:

Syphilis is a sexually transmitted infection (STI) caused by the spirochete *Treponema pallidum pallidum*. Recent years have seen a resurgence of syphilis. This is mainly due to an increased number of cases diagnosed in men who have sex with men (MSM).[1] Infection is associated with erratic condom use and a high number of sexual partners in the months before diagnosis.[2]

In Spain the incidence of syphilis has tripled since the beginning of this century, increasing from 1.96 to 5.70 per 100 000 population between 1998 and 2008..[1,3]

Even with effective treatment, the disease remains a significant public health problem with high associated economic costs.[2,3] Correct diagnosis is crucial in order to start antibiotic therapy as soon as possible and thus prevent new infections and stop the progression of the disease and associated sequelae.[4] Accordingly, new, more effective diagnostic techniques are required.

Traditionally the diagnosis of syphilis is based on a combination of clinical signs and symptoms, serological tests, and direct detection of the organism using dark field microscopy and silver impregnation techniques.[1,5,6] A definitive diagnosis can be reached only by direct detection of the microorganism in infected tissues.[7]

These diagnostic methods can yield conflicting results in many clinical situations. Obtaining the clinical history of a patient with an STI can be difficult due to feelings of embarrassment or guilt.[4] Moreover, syphilis has a wide range of clinical manifestations, which has earned it the epigram *the great imitator*. It can present with very atypical clinical features and hence be mistaken for genital ulcers or other different types of rash.[4–6,8] Clinical diagnosis thus depends on the experience of the physician and the presence of lesions.[5]

Serology, using a combination of nontreponemal tests (Venereal Disease Research Laboratory and rapid plasma reagin) and treponemal tests (fluorescent treponemal antibody absorption and *Treponema pallidum* particle agglutination assay), has become the most commonly used means of indirect diagnosis, with proven benefits for the diagnosis and monitoring of patients after the initiation of treatment, although several acute and chronic infections can produce false positives in nontreponemal tests While treponemal tests are highly specific and sensitive (almost 100%) in cases of secondary syphilis, sensitivity and/or specificity are lower in the early stages of infection, in cases of congenital and tertiary syphilis, neurosyphilis with HIV coinfection, and in other immunosuppressive conditions.[1,5,6,9] The increasingly common association of syphilis with HIV should be noted, given the confusion that can arise due to serological test results in these cases and the quite atypical clinical course.[5]

For direct detection of *T pallidum* dark field microscopy is sensitive but not specific, and requires trained personnel. Silver impregnation techniques are of low sensitivity (33% to 71%) and low specificity. Moreover, they give rise to numerous field artifacts and lack the necessary specificity to identify contaminant nontreponemal spirochetes (e.g., in oral mucosa).[5,6,9]

Table 1 Clinical and Serological Features of the 5 Clinical Cases.

Clinical Cases	Sex	Age	Clinical Features	HIV	ELISA	RPR	TPHA	Case Details	IHC
Case 1	M	42	Painful genital ulcer Swollen lymph nodes	+	+	1/4	—	TPHA	+
Case 2	M	54	Two painful genital ulcers Swollen lymph nodes	—	—	NP	NP	ELISA with typical clinical features	+
Case 3	M	37	Plaques with depressed center and multiple non-painful ulcers in the pubic area	—	—	NP	NP	ELISA with typical clinical features	+
Case 4	M	47	Faint macular rash without palmoplantar involvement	—	+	1/64	1/2560	Atypical clinical features	+
Case 5	F	55	Whitish papillary lesions on the lateral border of the tongue	—	NP	1/128	1/2560	Atypical clinical features	+

Abbreviations: ELISA, enzyme-linked immunosorbent assay; F, female; HIV, human immunodeficiency virus; IHC, immunohistochemistry; M, male; NP, not performed; RPR, rapid plasma regain; TPHA, *Treponema pallidum* haemagglutination test.

☆ Please cite this article as: Hernández C, Fúnez R, Repiso B, Frieyro M. Utilidad de la inmunohistoquímica con anticuerpos antitreponema en el diagnóstico de la sífilis. Actas Dermosifiliogr. 2013;104:926–928.

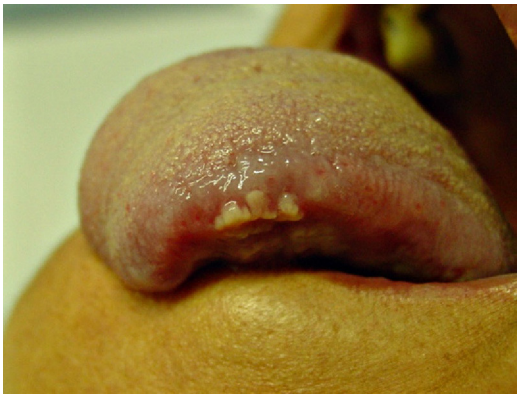

Figure 1 Case 5. Whitish papillary lesions on the lateral border of the tongue.

Several recently published findings support the role of biopsy with immunohistochemistry (IHC) using antitreponemal antibodies for the diagnosis of syphilis. One study comparing IHC with silver impregnation techniques concluded that the former was more sensitive.[8] Indeed, the sensitivity of IHC is over 90% in most cases, although lower values (71%) have been reported in some studies.[10] Sensitivity and specificity are highest in early primary and secondary syphilis (when spirochetes are found in large quantities), but diminish in later stages due to decreases in the number of microorganisms in the lesions.[5] IHC can distinguish syphilis lesions from other unrelated lesions, which may appear in patients with both true and false positive serology for *T pallidum*. Moreover, this technique permits the detection of spirochetes in the skin, although cross-reactivity with *Borrelia* species is possible.[4] Furthermore, IHC is a relatively rapid and inexpensive technique, with results available in about 48 h.

Here we present 5 clinical cases of patients treated in our STI outpatient clinic between January 2009 and January 2012 with a final diagnosis of syphilis (Table 1); in all cases IHC was crucial for accurate diagnosis. The selected cases are representative of those seen in everyday practice, in terms of their clinical manifestations and/or doubtful or even negative serology, and thus were difficult to diagnose. Case 1 was an HIV-positive patient with a negative treponemal test, a lesion suggestive of syphilitic chancre, and no lymph node enlargement. This test result may have been due to the window period, or may reflect a false negative influenced by HIV co-infection. In case 2, the patient had a lesion suggestive of syphilitic chancre, but a negative enzyme-linked immunosorbent assay (ELISA), which was also probably due to the window period. Similarly, the negative ELISA in case 3, despite lesions suggestive of secondary syphilis, was probably a rare case of a false negative using this technique. The patient in case 4 presented rarely seen clinical features with a faint macular rash without palmoplantar involvement. Finally, case 5 (Figs. 1 and 2) is an example of atypical extragenital clinical manifestations.

IHC offers increased sensitivity and specificity compared with conventional techniques for the detection of *T pallidum*.[3–6,8–10] Although this technique is not a widely used diagnostic method and we do not recommend its routine use, these 5 cases highlight its utility, especially in HIV patients or immunosuppressed patients in general, in those with secondary syphilis with negative and/or dubious serology, in cases with atypical clinical features (including cases of extragenital syphilis), and in the early stages of the disease.

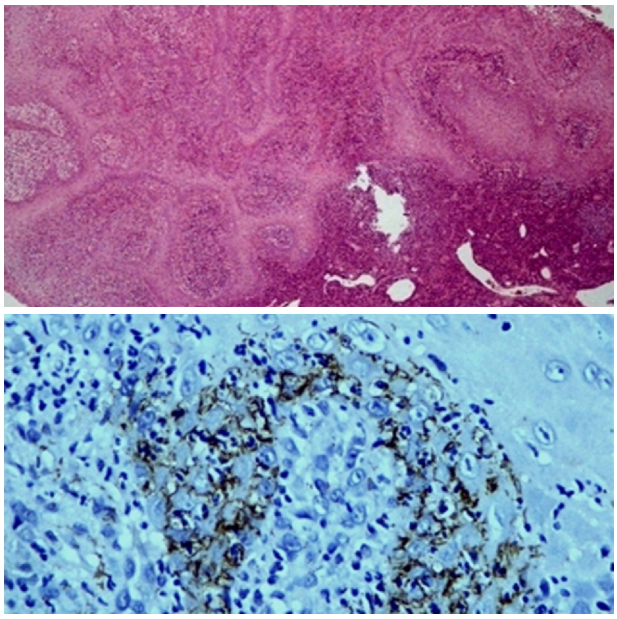

Figure 2 Biopsy of the lesion shown in Figure 1. Upper image: intense inflammatory infiltrate with a predominance of plasma cells (hematoxylin-eosin, original magnification × 50). Lower panel: IHC of the same biopsy (original magnification × 400) showing infiltration of treponemata, which appear as brownish bead-like structures.

References

1. Fuente MJ. El resurgir de la sífilis. Actas Dermosifiliogr. 2010;101:817–9.
2. Repiso B, Frieyro M, Rivas-Ruiz F, de Troya M. Uso del preservativo y número de parejas sexuales en hombres que tienen sexo con hombres con sífilis. Actas Dermosifiliogr. 2010;101:847–52.
3. Perna C, Cuevas J, Hardisson D, García Fernández E, Beato MJ, Contreras F. Valoración anatomopatológica e inmunohistoquímica frente a *Treponema pallidum* en 42 biopsias con sospecha clinicopatológica de sífilis. Rev Esp Patol. 2011;44:145–50.
4. Quatresooz P, Piérard GE. Skin Homing of *Treponema pallidum* in early syphilis, an immunohistochemical study. Appl Immunohistochem Mol Morphol. 2009;17:47–50.
5. Müller H, Eisendle K, Bräuninger W, Kutzner H, Cerroni L, Zelger B. Comparative analysis of immunohistochemistry, polymerase chain reaction and focus-floating microscopy for the detection of *Treponema pallidum* in mucocutaneous lesions of primary, secondary and tertiary syphilis. Br J Dermatol. 2011;165:50–60.
6. Hoang MP, High WA, Molberg KH. Secondary syphilis: a histologic and immunohistochemical evaluation. J Cutan Pathol. 2004;31:595–9.
7. Sexually transmitted diseases treatment guidelines. Centers for Disease Control and Prevention. MMWR. 2010;59:26-40. [consultado 1 Mar 2012]. Disponible en: http://www.cdc.gov/std/treatment/2010/STD-Treatment-2010-RR5912.pdf

8. Phelps RG, Knispel J, Schuman Tu E, Cernainu G, Saruk M. Immunoperoxidase technique for detecting spirochetes in tissue sections: camparison with other methods. Int J Dermatol. 2000;39:609–13.
9. Buffet M, Grange P, Gerhardt P, Carlotti A, Calvez V, Bianchi A, et al. Diagnosing *Treponema pallidum* in secondary syphilis by PCR and immunohistochemistry. J Invest Dermatol. 2007;127:2345–50.
10. Martín-Ezquerra G, Fernández-Casado A, Barco D, Jucglà A, Juanpere-Rodero N, Manresa JM, et al. *Treponema pallidum* distribution patterns in mucocutaneous lesions of primary and secondary syphilis: an immunohistochemical and ultrastructural study. Human Pathol. 2009;40:624–30.

C. Hernández,[a,*] R. Fúnez,[b] B. Repiso,[a] M. Frieyro[a]

[a] *Servicio de Dermatología, Hospital Costa del Sol, Marbella, Spain*
[b] *Servicios de Anatomía Patológica, Hospital Costa del Sol, Marbella, Spain*

* Corresponding author.
E-mail address: chernandez@aedv.es (C. Hernández).

Successful Treatment of Leiomyoma of the Nipple With Carbon Dioxide Laser☆

Buenos resultados tras tratamiento con láser CO_2 de leiomioma en el pezón

To the Editor:

Genital leiomyomas are the least common type of leiomyomas and those located on the nipple or areola are extremely rare.[1–4] To date, their treatment with carbon dioxide (CO_2) laser has not been reported in the literature.

We describe the case of a 33-year-old woman with no relevant past medical history who presented with a skin lesion on the left nipple; the lesion had been stable since its appearance in adolescence. The patient reported mild tenderness and great impact on personal appearance as the reasons for seeking treatment. The examination revealed elongation of the left nipple secondary to the presence of a well-defined, firm nodular lesion that was the same color as the nipple and about 15 mm in diameter; there were no signs of nipple retraction (Fig. 1). The rest of the left breast and the right breast revealed no significant findings.

Histopathology of the lesion revealed a hyperkeratotic epidermis with increased pigmentation of the basal layer and, in the dermis, the presence of a poorly defined, unencapsulated tumor composed of multiple interwoven bundles of smooth muscle formed by cells with ovoid nuclei and eosinophilic cytoplasm (Fig. 2A). No atypical cells or mitoses were observed. Staining for smooth muscle actin was positive (Fig. 2B). In view of these clinical and histologic findings, the lesions was diagnosed as a genital leiomyoma. Under local anesthesia with mepivacaine 2%, the rest of the lesion was removed with a Sharplan CO_2 laser in unfocused mode with an irradiance of 55 W/cm^2 and a spot size of 3 mm. The lesion was treated in a single session with 5 to 8 passes; between each pass the coagulated tissue was removed until there was no visible sign of the leiomyoma. After 1 treatment session much of the lesion had been removed with good cosmetic and functional results. No recurrence was detected 20 months after treatment (Fig. 3).

Cutaneous leiomyomas are rare, benign tumors. They are classified into 3 categories according to the muscle fibers from which they originate: piloleiomyoma, originating from the smooth muscle fibers of the arrector pili muscle; angioleiomyoma, originating from the tunica media of the blood vessels; and dartoic or genital leiomyoma, originating from the smooth muscles of the scrotum, vulva, nipple, or areola. Genital leiomyomas are the least common type and those located on the nipple or areola are extremely rare.[1–4] Leiomyomas of the nipple and areola, first described in 1854 by Virchow, present as small, solitary nodular lesions covered with healthy skin that can exceptionally cause nipple retraction and spread to the underlying breast tissue.[4] The lesions are usually asymptomatic but in rare cases can cause pain spontaneously or in response to stimuli such as cold, pressure, or emotional stress.

Histologically, they are poorly defined, unencapsulated skin tumors composed of bundles of spindle cells with abundant eosinophilic cytoplasm and elongated nuclei with blunt ends, arranged in an interwoven pattern. The epidermis is usually unaffected because it is separated from the tumors by a free zone of dermis; cellular mitosis and atypia are

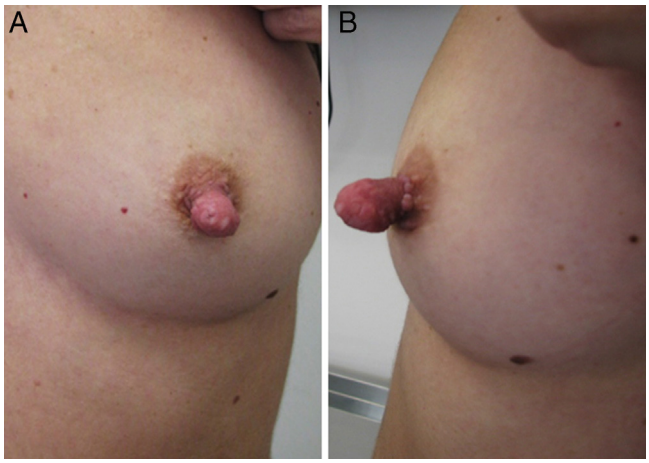

Figure 1 Skin lesion located on the patient's left nipple.

☆ Please cite this article as: López V, López I, Alcacer J, Ricart JM. Buenos resultados tras tratamiento con láser CO_2 de leiomioma en el pezón. Actas Dermosifiliogr. 2013;104:928–930.