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Use of PCR and MLST analysis to establish *Treponema pallidum* as the cause of a painful oral lesion

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BACKGROUND

Since 2001, when primary and secondary (P&S) syphilis reached a historic low in the United States, the case rate of syphilis has increased almost every year. In 2019, 129,813 cases of syphilis were reported, including 38,992 P&S cases [1]. Orange County (OC), located 60 miles north of New York City (NYC), surpasses this trend and has seen an 88% increase in syphilis cases between 2017 and 2021 [2]. In 2021, OC was listed as one of the top five counties in New York State outside of NYC, with increasing diagnoses of early and congenital syphilis [3].

Prompt diagnosis and treatment of syphilis are crucial to controlling the spread. Although clinicians have been trained to diagnose primary syphilis with the identification of a chancre, classically described as an indurated, painless ulcer at the site of *Treponema pallidum* inoculation, several case series have included patients who had tender lesions, single or multiple, without evidence of concurrent *Herpes simplex virus* (HSV) infection to explain the pain. A reasonable question to ask is whether the presence of a painful chancre is due to infection caused by a particular strain (or strains) of *T. pallidum*.

CASE PRESENTATION

A 39-year-old heterosexual, monogamous, HIV-negative woman presented with a painful tongue lesion of one month's duration. A possible tongue malignancy with a superimposed bacterial infection was hypothesized. The lesion was biopsied, and treatment with oral clindamycin was initiated, but it did not improve.

The tissue histopathology revealed an intense inflammatory reaction with a predominance of lymphocytes, plasma cells, and CD68+ histiocytes. *Treponema pallidum* immunocytochemistry demonstrated a high density of spirochetes in the subepithelial connective tissue.

Serologic testing for syphilis demonstrated a reactive *anti-Treponema pallidum* IgG EIA and an RPR titer of 1:8.

PCR amplification and multi-locus sequence typing (MLST) [4] confirmed that the visualized spirochetes were *T. pallidum*. This particular MLST strain pattern has been identified twice before and named ST-109, but no clinical information is available regarding these prior strains.

Diagnosis and appropriate treatment were delayed since syphilis was not suspected initially. Changing the treatment from clindamycin to doxycycline led to improvement of the tongue lesion. When the pathology and syphilis serology results became available, the patient was treated with 2.4 million units of benzathine penicillin given intramuscularly with complete resolution of the lesion.

Figure 1. Photography of tongue and lesion before and after seven days of treatment with oral Doxycycline. On the left, the area of biopsy can be appreciated.

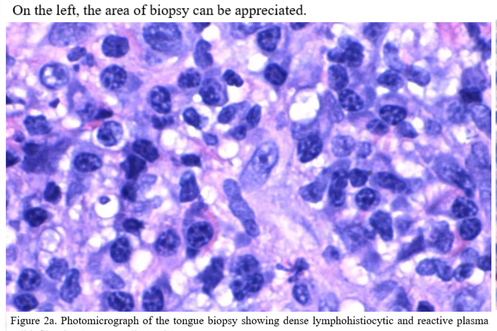

Figure 2a. Photomicrograph of the tongue biopsy showing dense lymphohistiocytic and reactive plasma cell infiltration (Hematoxylin-Eosin).

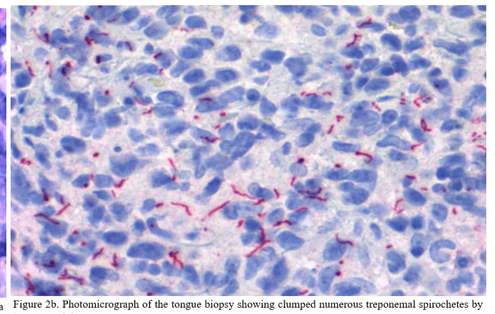

Figure 2b. Photomicrograph of the tongue biopsy showing clumped numerous treponemal spirochetes by immunostaining.

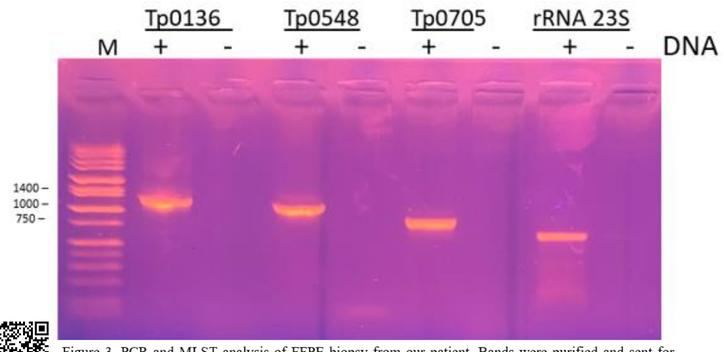

Figure 3. PCR and MLST analysis of FFPE biopsy from our patient. Bands were purified and sent for sequencing; all were T. pallidum sequences.

The male partner, who reported no symptoms, had a positive *T. pallidum* EIA with an RPR titer of 1:64 and was also treated with penicillin.

Within four months, a woman from an adjacent town presented to urgent care with three perineal severely painful lesions, testing negative for HIV, HSV by PCR of an ulcer swab, and chlamydia and gonorrhea on a urine sample by a NAAT. The anti-*T. pallidum* EIA was reactive, and the RPR titer was 1:4. The lesions resolved with IM penicillin treatment.

UPDATES

Since the submission of the abstract, other cases of painful lesions attributed to syphilis, based on presentation and serologies, have been found in OC. Only women seem to be affected, while the male partners remain asymptomatic.

DISCUSSION

Despite several published reports of painful syphilitic chancres, clinicians continue to rely on the classic painless presentation when diagnosing patients with syphilis. Ignoring atypical painful presentations can lead to a delay in diagnosis and treatment.

FFPE tissue samples are challenging for DNA extraction, often resulting in low yields. Even though DNA extraction was considered adequate in this case, in the future, we suggest sending biopsy samples directly for sequencing.

CONCLUSIONS

A subset of early syphilis cases may present with painful lesions.

Given the rise in the number of cases of syphilis in the USA, it is essential that clinicians be aware of this atypical presentation.

It remains to be determined whether such painful lesions are only associated with certain T. pallidum genotypes.

FUTURE STUDIES

As a result of this case, there is an ongoing plan to initiate retrospective and prospective studies in conjunction with the OCDOH and the CDC. Whole genome sequencing was obtained for this case, and for other suspected cases, sequencing is ongoing.

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