Published in final edited form as:

Am J Dermatopathol. 2024 January 01; 46(1): 31–35. doi:10.1097/DAD.0000000000002583.

Round Bodies Detected by *Treponema pallidum* Immunohistochemical Stain in Two Cases of Cutaneous Syphilitic Gummata

Suzanne W. Birmingham, MD[*], **Lina Saeed, MD**[*], **Charles M. Thurlow, PhD**[†], **Kendra Vilfort, BS**[†], **Allan Pillay, PhD**[†], **Nathan W. Rojek, MD**[*], **Linda T. Doan, MD**[*], **Bonnie A. Lee, MD**[*]

[*]Department of Dermatology, University of California, Irvine, Irvine, CA;

[†]Division of STD Prevention, Centers for Disease Control and Prevention, Atlanta, GA.

Abstract

Tertiary syphilis may present a diagnostic challenge due to negative nontreponemal serologies in up to 30% of cases and frequent lack of identifiable spirochetes on histopathology or other direct detection tests. We report 2 cases of round bodies staining with *Treponema pallidum* immunohistochemistry by light microscopy in biopsies from cutaneous syphilitic gummata. In 1 case, the finding was validated 3 times by 2 independent laboratories; in the other case, *T. pallidum* was detected by polymerase chain reaction in the biopsy sample. Spirochete round bodies have previously been reported in the setting of electron microscopy and fluorography, but to the best of our knowledge, have not been reported by light microscopy in a routine skin biopsy. Although the clinical implications are unclear, this may represent a helpful new paradigm for the diagnosis of tertiary syphilis.

Keywords

syphilis; immunohistochemistry; PCR; spirochetales; treponemal infections

INTRODUCTION

Syphilis refers to a broad spectrum of clinical findings caused by infection with the spirochete *Treponema pallidum* subspecies *pallidum* (hereafter referred to as *T. pallidum*).

Known as “the great imitator,” syphilis may present in a wide variety of ways, often determined by the chronicity of disease (Table 1). One of the most classic presentations of tertiary syphilis is the gumma, a granulomatous response to *T. pallidum* infection, which may affect any organ system. Clinically, cutaneous syphilitic gummata typically present as asymptomatic ulcers with central necrosis. They may rapidly progress to several centimeters in size, especially in the setting of HIV. Biopsies from syphilitic gummata

Correspondence: Suzanne W. Birmingham, MD, 1701 Divisidero Street, San Francisco, CA 94115 (Suzanne.c.ward.1@gmail.com).

The authors declare no conflicts of interest.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

classically demonstrate epithelioid granulomas with caseation and brisk lymphoplasmacytic infiltrate.[1,2]

Diagnosis of tertiary syphilis can, on occasion, be very difficult. Treponemal antibodies are a sensitive indicator of infection in tertiary syphilis as with other stages. However, their presence alone is not sufficient for diagnosis given that, once exposed, treponemal antibodies usually remain positive for life regardless of treatment. In addition, up to 30% of patients with tertiary syphilis lose reactivity in nontreponemal tests (such as Venereal Disease Research Laboratory), which poses challenges for disease diagnosis.[3] Spirochetes are often rare or absent with direct visualization using Warthin–Starry stain or darkfield microscopy as tertiary syphilis is largely immune-mediated with a relative scarcity of *T. pallidum* organisms.[1,9,10] *T. pallidum* immunohistochemistry (IHC) staining and polymerase chain reaction (PCR) have also both failed to act as a sensitive confirmatory test in tertiary syphilis, with 1 study demonstrating 15% and 14% sensitivity, respectively.[11] Despite these pitfalls, identification of rare spirochetes on histopathologic review is often key for establishing a diagnosis in these challenging cases.

In the past, the helical shape of spirochetes has been an important consideration in evaluating the positivity of a treponemal IHC stain. Although “round bodies” (also known as “cyst forms”) have been described in *T. pallidum* and other spirochetes, these structures have been identified only with fluorography and electron microscopy to date.[12–16]

In the following report, we describe 2 cases of round bodies evident by light microscopy with a treponemal IHC stain.

REPORT OF CASES

Patient 1 was a 52-year-old man with a history of HIV (not currently taking highly active antiretroviral therapy) and partially treated late latent syphilis who presented to the emergency department with a 1-week history of painless ulcer on the back. He denied similar lesions in the past but did note a history of third-degree burns in the area, status post–split-thickness skin grafts. The patient received broad-spectrum antibiotic coverage with vancomycin and piperacillin–tazobactam before evaluation by the dermatology service. Physical examination revealed a four-centimeter ulcer with a cribriform border, fibrinous base, and surrounding violaceous rim on the left upper back (Fig. 1A).

Laboratory evaluation was remarkable for $CD4^+$ T-cell count of 237/μL (reference range 477–1634/μL), HIV viral load of 111,951 copies/mL (reference range not detected), and positive treponemal antibody screen (Chemiluminescent Immunoassay, DiaSorin, Vercelli, Italy) with rapid plasma reagin titer of 1:8 (reference range <1:1). Tissue culture isolated 2 morphotypes of Gram-negative rods, beta hemolytic *Streptococcus*, and few oxacillin-resistant *Staphylococcus aureus*. Serologic testing for viral and fungal etiologies was negative.

Broad shave biopsy was obtained, which revealed an ulcer with dense lymphoplasmacytic infiltrate and focal suppurative and granulomatous inflammation (Figs. 1B–F). Fite, acid-fast bacillus, Grocott methenamine silver, and Gram stains failed to demonstrate additional

organisms. Spirochete immunostaining showed round, dot-like structures (Figs. 1C–F). Given the unusual findings, IHC was repeated at the same laboratory and then sent to a second laboratory for repeat testing, and all 3 studies demonstrated identical features. Of note, both laboratories used an anti-*T. pallidum* rabbit polyclonal antibody from Biocare Medical (Pacheco, CA).

The patient was diagnosed with cutaneous gumma of tertiary syphilis, complicated by polymicrobial skin infection. He was treated with broad-spectrum antimicrobials, including intramuscular benzathine penicillin G, and was urged to resume antiretroviral therapy. Unfortunately, he was lost to follow-up.

Patient 2 was a 36-year-old man with a history of substance use disorder who presented to the emergency department with a three-month history of tender, nonhealing wounds on the bilateral palms. He had been unsuccessfully treated with multiple rounds of topical and oral antibiotics at outside facilities, including mupirocin ointment, triple antibiotic ointment, oral clindamycin, oral doxycycline, and intramuscular ceftriaxone. Review of systems was remarkable for progressive blurry vision for the past year and subjective hand weakness. Physical examination revealed 2–3 cm vegetating ulcers on the bilateral palms (Fig. 2A).

Treponemal antibody screen was positive and rapid plasma reagin titer was 1:256. HIV screen was negative. Tissue culture revealed few diphtheroids and 3 morphotypes of anaerobic Gram-positive cocci. Punch biopsy was obtained from the friable wound edge and revealed dermal necrosis and fibrosis. Spirochete stain revealed both helical spirochetes and, once again, round structures (Figs. 2B, C). Gram stain revealed gram-negative cocci. Fite and Grocott methenamine silver stains were negative. He left the hospital against medical advice before a lumbar puncture was obtained.

The patient was diagnosed with tertiary syphilis based on positive serologies, cutaneous lesions clinically and histopathologically consistent with gummata (although no granulomatous inflammation was identified given degree of necrosis), and review of systems potentially concerning for neurologic involvement. He was treated with 2.4 million units of intramuscular benzathine penicillin G and urged to follow-up with the Department of Public Health for additional treatment and repeat serologies. Two months later, the palmar lesions had healed.

Formalin-fixed paraffin-embedded tissue blocks from each case were sent to the Centers for Disease Control and Prevention for *T. pallidum* PCR using a research use only test. DNA was extracted from the formalin-fixed paraffin-embedded tissue specimens using the Qiagen DNA Mini Kit (Qiagen, Germantown, MD), and a real-time duplex PCR targeting the DNA polymerase I gene (*polA*) of *T. pallidum* and human RNase P gene was performed as previously described with slight modifications.[17] In brief, 6-carboxyfluorescein and Quasar 670 dyes were used for the *polA* and RNase P TaqMan probes, respectively. The PCR mixture consisted of 25 μL of PerfectA qPCR Supermix (Quantabio, Beverly, MA) and 20 μL of DNA in a 50 μL reaction. Appropriate positive and no template controls were included in the runs. PCR was performed in a Rotor-Gene Q real-time PCR instrument (Qiagen). Although PCR was negative in case 1, *T. pallidum* was detected in the tissue from case 2.

DISCUSSION

Ovcinnikov and Delektorskij first reported syphilis round bodies on electron microscopy in 1968.[12] Although relatively little research has been performed with *T. pallidum* round bodies beyond documenting their existence, there has been quite a bit of investigation into round bodies associated with its fellow spirochete, *Borrelia burgdorferi*. It stands to reason that the 2 spirochetes may assume this temporary circular configuration under similar circumstances. In a study of antibiotic effect on spirochete morphology in vitro, surviving spirochetes assumed a round, nonmotile form after exposure to tigecycline, which was reversible 1 week after antibiotic discontinuation.[18] Electron microscopy of these bodies revealed the presence of viable flagella and core structures within an outer membrane. This process seems to be dose-dependent as very low concentrations of tigecycline did not induce round body formation, whereas very high concentrations were cytotoxic to the spirochetes. *Borrelia burgdorferi* round bodies have also been reported in the setting of aging, complement, temperature changes, and growth medium changes such as alkaline pH or serum starvation.[14,16,18,19] In both patients presented, antibiotics administered less than 7 days before biopsy may have driven formation of spirochete round bodies. Although the clinical implications of round bodies in Lyme disease have been somewhat controversial and their role in “chronic Lyme disease” has been widely discredited, the topic is outside the scope of this report.[20] The clinical significance of such bodies in syphilis remains unknown.

As with any stain, *T. pallidum* IHC must be interpreted with some degree of subjectivity. Past reports raise concern about the specificity of the stain, which may be positive in the presence of other spirochetes (such as *B. burgdorferi* and *Helicobacter pylori*) or even nontuberculous mycobacteria.[21–23] As such, it is standard practice to disregard structures that are not helical in shape and to consider other bacteria in the spirochete family or obtain additional stains such as Fite and acid-fast bacillus when clinically relevant.

In case 1, a false-positive IHC result would seem unlikely in this patient with positive syphilis serologies, a clinical presentation consistent with syphilitic gumma, and otherwise negative workup. Furthermore, the consistent presence of these round structures on 3 studies obtained at 2 different laboratories, with minimal background staining, argue against the possibility of artifact. Despite the compelling history and staining, PCR testing of skin formalin-fixed paraffin-embedded tissue for *T. pallidum* was negative. Given the documented low sensitivity of PCR for tertiary syphilis and biopsy sampling postantibiotic treatment, this result was not unexpected.[24] Syphilis remains the most likely etiology in patient 1 given these clinical and laboratory findings. In case 2, round bodies appear side by side with traditional helical spirochetes, again with minimal background staining. It is somewhat atypical for gummata to be tender; however, this may be explained by the concomitant bacterial infection. Positive serologies and tissue PCR further support a diagnosis of tertiary syphilis. Given PCR was negative in case 1 (only round bodies visualized on IHC) and positive in case 2 (round bodies and traditional spirochetes visualized on IHC), we wonder whether conversion to round bodies combined with clearance of *T. pallidum* due to antibiotic treatment altered the sensitivity of PCR. Further testing is needed to better assess the prevalence and clinical implications of round bodies in syphilis.

To the best of our knowledge, these are the first reported cases of *T. pallidum*-staining round bodies evident on light microscopy. In the setting of tertiary syphilis, in which the presence of spirochetes is rare and diagnosis is often difficult, recognition of this form may prove a valuable tool for pathologists and clinicians alike.

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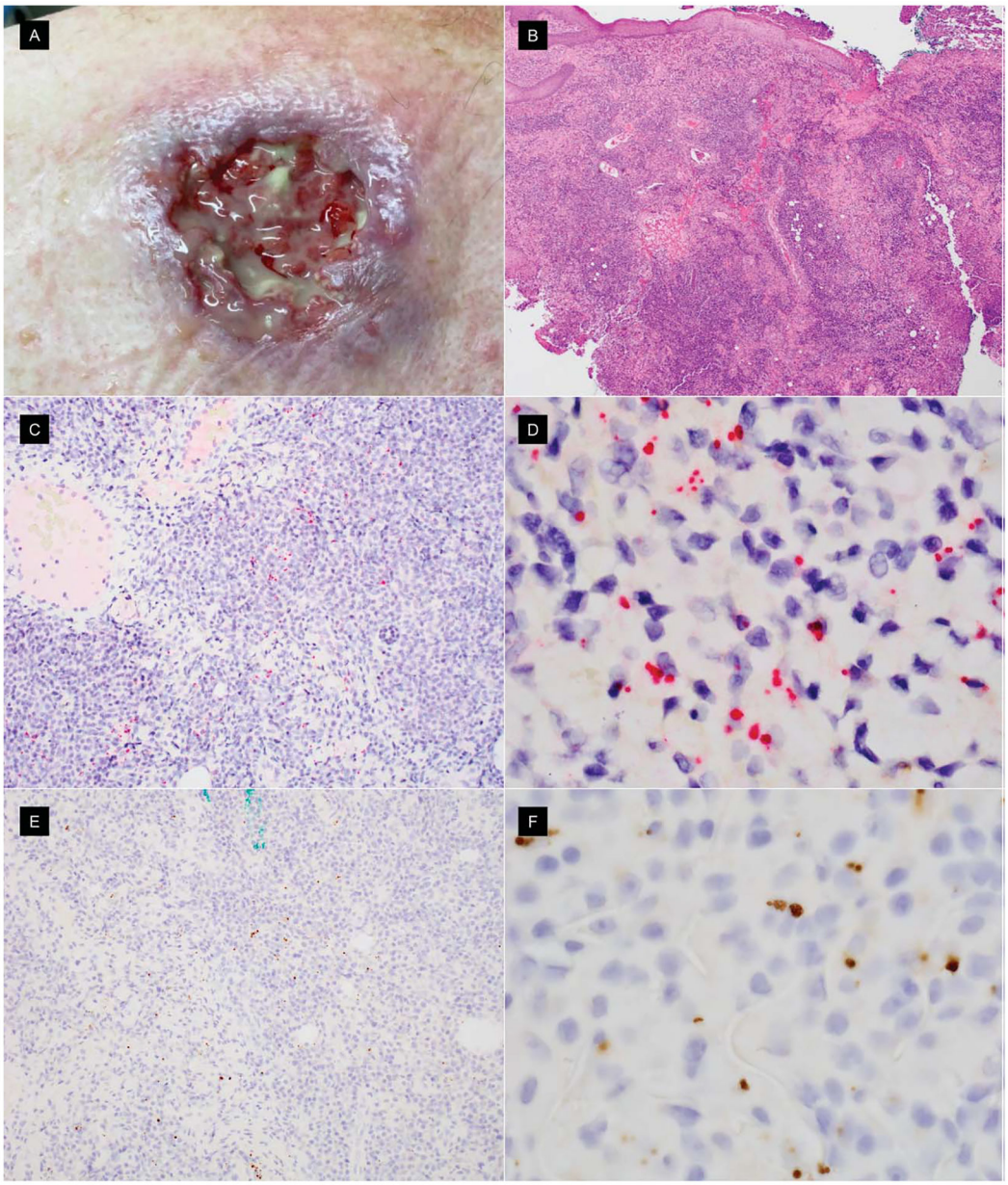

FIGURE 1.

Patient 1 syphilitic gumma, clinical and histopathologic images. A, 4-cm ulcer with irregular border and violaceous rim. B, Ulcer with dense lymphoplasmacytic infiltrate (H&E, ×40). C, Discrete round-shaped staining bodies (*T. pallidum* IHC stain performed at APMG, ×200). D, Round bodies at high magnification (*T. pallidum* IHC stain, APMG, ×1000). E, Discrete round-shaped staining bodies (*T. pallidum* IHC stain performed at NeoGenomics, ×200). F, Round bodies at high magnification (*T. pallidum* IHC stain, Neogenomics, ×1000). APMG, Affiliated Pathologists Medical Group.

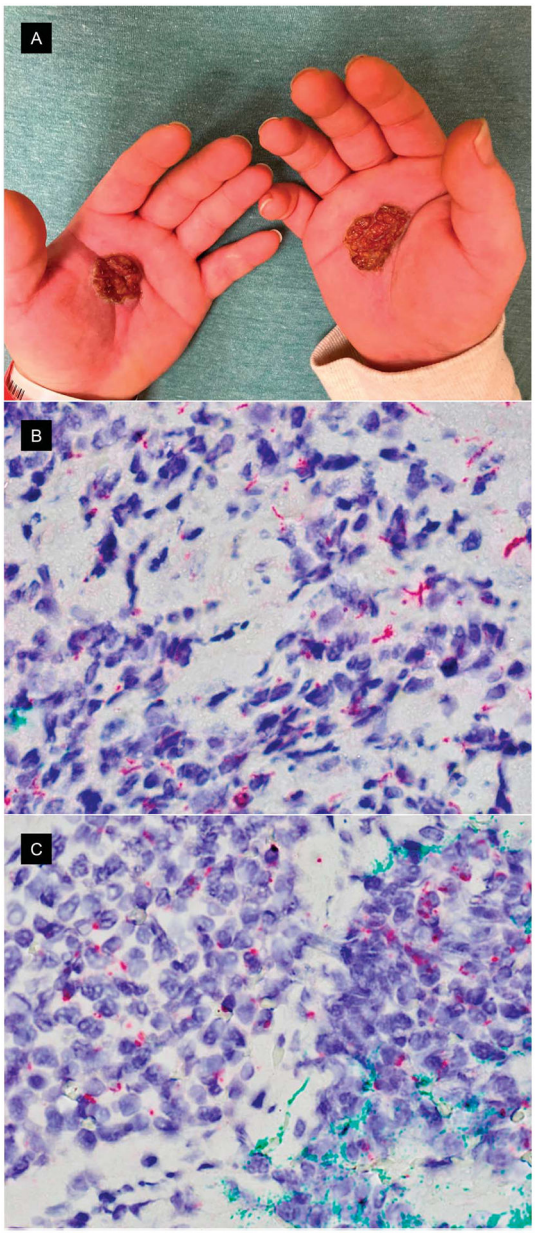

FIGURE 2.

Patient 2 syphilitic gumma, clinical and histopathologic images. A, 2 cm vegetating ulcers on the bilateral palms. B, Traditional helical spirochete forms at high magnification (*T. pallidum* IHC stain, APMG, ×1000). C, Discrete round bodies at high magnification (*T. pallidum* IHC stain, APMG, ×1000).

TABLE 1.

Overview of Systemic and Cutaneous Manifestations of Syphilis Based on Stage[3–7]

Stage	Natural History	Extracutaneous Manifestations	Cutaneous Manifestations	Histopathologic Appearance	Serologic findings *
Primary syphilis	Chancre appears 2–3 weeks after exposure. Heals without scarring in 3–6 weeks if untreated	Localized lymphadenopathy	Painless chancre	Ulcer with underlying dense perivascular lymphocytic infiltrate often with plasma cells	TT positive NTTs reactivity increase throughout primary infection
Secondary syphilis	Presents 6–8 weeks after chancre. Can heal without scarring after weeks–months if untreated	Diffuse lymphadenopathy Hepatosplenomegaly Hepatitis Nephrotic syndrome Aseptic meningitis	Skin rash (polymorphic but classically copper colored macules involving the palms and soles) Mucous patches Condyloma lata	Vacuolar interface dermatitis, often with psoriasiform epidermal hyperplasia, endothelial swelling, perivascular and/or interstitial inflammatory infiltrate often with plasma cells	TT positive NTT reactivity at peak, risk of false negative due to prozone effect at extremely high titers
Latent syphilis	Early latent syphilis defined as the first year after resolution of secondary syphilis After 1 year, it is termed late latent syphilis	None	None	NA	TT positive NTT reactivity begins to decline
Tertiary syphilis	Years to decades after initial infection	Cardiovascular: aortic aneurism, valvular disease, coronary artery disease Gummatous disease (may affect any organ)	Superficial nodular lesions Mucocutaneous gummata	Mixed-dense inflammatory infiltrate with vascular proliferation Gumma: Granuloma ± central necrosis with mixed inflammatory infiltrate and fibrosis	TT positive NTTs may be negative in up to 30% of cases
Neurosyphilis	Can present at any stage	General paresis Tabes dorsalis Uveitis Cranial nerve palsies Stroke	NA	NA	TT positive CSF VDRL reactive

* NTTs (eg, rapid plasma reagin and VDRL) are sensitive screening tests, but are prone to false-positive results in as many as 18% of patients.[8] Positive NTT should be followed with confirmatory TT (eg, *T. pallidum* particle agglutination assay). TTs are up to 99% specific for syphilis but usually remain positive for life after primary infection.[4]

CSF, cerebrospinal fluid; NA, not available; NTT, nontreponemal test; TT, treponemal test; VDRL, Venereal Disease Research Laboratory.