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False Positive Versus False Negative in Neonate Suspected Congenital Syphilis, Case Report with Analysis for Quality Improvement

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Introduction

Treponema pallidum subspecies *pallidum* is a pathogenic spirochete responsible for syphilis, a sexually transmitted infection with a characteristic clinical presentation that alternates from active to dormant stages with progressively increased severity (9). These stages are classified as primary, secondary, and tertiary syphilis; each stage has unique findings on history and physical exam (7). The *T. Pallidum* subspecies that cause syphilis is uniquely transmitted by sexual contact, while other subspecies of treponemes are transmitted by close nonvenereal contact (9).

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T. pallidum varies from 6 to 15 µm in length and is 0.2 µm in diameter, which is relatively thin compared to other prokaryotic organisms. The spiral-shaped body is surrounded by a dual membrane system: a cytoplasmic membrane, a loosely associated outer membrane, and a thin layer of peptidoglycan between each of the membranes, analogous to gram-negative bacteria. One unique feature of this membrane is the peptidoglycan layer compared to gram-negative bacteria is its more proximal association with the inner membrane, whereas gram-negative bacteria's peptidoglycan layer is firmly linked to both membranes (5). This structural difference is theorized to be why penicillin antibiotics, which inhibit peptidoglycan linkage, are so efficacious in treating *T. pallidum* (5) (9). The endoflagella organelle is located in the periplasmic space giving *T. pallidum* a corkscrew pattern motility. It is important to note that the motility of *T. pallidum* is a major virulence factor, as it allows the dissemination of infection and seeding of multiple unrelated organ sites. This includes immune-privileged sites like the eye and epididymis, where replication can occur unimpeded by the immune system (5),(9).

Ongoing research suggests that *T. pallidum* has multiple mechanisms that it utilizes to successfully infect and propagate within a host for an extended period while avoiding eradication by the host immune system. Proteins expressed on its surface membrane allowing attachment to host cell membranes and the extracellular matrix, resulting in successful host infection after initial exposure (5). *T. pallidum* then evades detection of the immune system via a slow cycle of replication which maintains a low antigenic threshold. It also evades detection by expressing very few antigenic proteins on its outer membrane surface and altering the epitopes of the more immunogenic antigens periodically through facilitated rearrangements of the TpRK gene (5). These factors allow *T. pallidum* to evade host immune detection and immune response, resulting in an anomalous cluster of non-specific symptoms or an entirely sub-clinical presentation (5).

Syphilis is called "the great imitator" colloquially (9) because these non-specific clinical findings are also seen in other, more common diseases deciding to test for the condition less likely. There are also many limitations with clinical testing for *T. pallidum*. There is no viable method of culturing *T. pallidum* outside of the host for diagnostic testing because the organism lacks any capacity to replicate outside of a viable host (4). *T. pallidum* nearly exceeds the limits of what can be resolved with routine light microscopy due to its thin spirochete morphology, and its unique cell wall makes visualization with gram staining a challenge. Researchers can detect treponeme samples with darkfield microscopy, silver stain microscopy, and electron microscopy; but accurately ruling out syphilis in this manner requires tissue sampling of an area with active signs of infection, and it is therefore not a good diagnostic tool for detecting T pallidum during the latent phases of infection (4).

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Given the limitations of conventional detection methodology, the predominant tests for T pallidum are either serological: evaluating the host for the production of antibodies against *T pallidum* antigens, or non-serological: indirect markers found to be elevated in association with *T. pallidum* infection (4). One challenge this testing modality presents is the fluctuation of the marker values due to disease dormancy and cross-reactivity of these testing modalities with other disease states such as falsely elevated RPR in association with autoimmune antiphospholipid syndromes (4, 7).

Difficulties with diagnosis and detection and the stigmatization of testing for sexually transmitted infections contribute to a higher prevalence of cases in the general population despite many efficacious treatments. In 2019, the US had 129,813 cases of all stages of syphilis reported, including 38,992 primary and secondary infections (6). This number has been continually increasing since 2001, with a 10% increase from 2018-2019 alone (6). Al-

though MSM populations are the most at risk for infection, there has been a marked increase in heterosexual populations, specifically the ratio of infected women (6).

Syphilis is a relevant disease in neonatology because *T. Pallidum* may transmit from mother to the immune-compromised fetus trans-placentally during pregnancy, causing a congenital infection reminiscent of late-stage syphilis and potentially fetal demise (7). The risk of this occurring is the highest during the active secondary phase of infection and lowest during the latent phase. The long-term consequences of an undetected and untreated maternal infection justify universal testing of all mothers prior to labor for syphilis (1, 7). Clinical serologic testing such as the CIA/EIA test is better suited to screen general population members, and non-serologic testing confirms the presence of infection due to each test's specificity and sensitivity relative to the general population. Serology is the more sensitive test, while non-serology is more specific for the presence of infection (4). If both tests results are positive, according to current CDC guidelines, the mother should receive penicillin G or an equivalent non-teratogenic antibiotic therapy by Intramuscular route for ten days and complete the course at least 30 days prior to delivery (1). Suppose this standard of care is not met. In that case, the neonate falls into either the category of "suspected" or "probable" congenital syphilis and should be treated appropriately with laboratory workup and Penicillin G IV. If there is a need for IV intervention and workup, the neonate will likely be admitted for care for the ten days following due to the more complex standard of care required for IV drugs in neonates (1).

The standard of care for "suspected" and "probable" congenital syphilis involves additional testing with Blood cell and platelet studies, electrolyte status evaluation, assessment of long bones for abnormalities via X-ray, serology studies, ophthalmologic evaluation of eyes for chorioretinitis and corneal keratitis, evaluation for rash or mucocutaneous lesions, assessment of liver and spleen size for possible hepatomegaly/hepatosplenomegaly, a lumbar puncture to assess for spirochetes invasion of the CNS and chest X-ray to evaluate for pneumonia alba or other abnormal findings (1). Guidelines recommend an additional confirmatory serology test for the mother with lower clinical suspicion to rule out false-positive findings (1-7). However, current medical literature guidelines do not provide much guidance regarding treating a patient who has a negative repeat serology test in conjunction with a positive serology finding prior. The current standard of practice is to treat both mother and neonate as per CDC guidelines regardless of clinical suspicion or follow-up serology testing results. This ultimately begs why additional testing is done if it provides no change to the course of treatment.

This case report summarizes the events of a patient encounter where this occurred while retrospectively assessing the clinical course. A protocol for hospitals to implement to address indeterminate testing events and what goals should be met before discharge to mitigate psychological stress caused by a false positive test for a stigmatized sexually transmitted infection is provided.

Patient Case

History:

Patient H (baby) was an infant of Hispanic descent born prematurely at 36 1/7 weeks EDC to a 37-year-old G5 P2 mother with gestational diabetes mellitus. She admitted to a rash during her pregnancy and a history of sudden fetal demise ending her last pregnancy. Prenatal testing showed positive RPR and EIA serology test results, and the patient's mother was diagnosed with probable syphilis. Other findings of the congenital infection panel were negative; Group B Strep rapid test was negative. Both mother and baby were Rhesus positive, and Coombs test was negative. The mother received one injection of penicillin G by Intramuscular route but could not complete treatment as per CDC guidelines due to her entering labor prematurely, which quickly progressed to vaginal delivery.

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Physical Exam and Test Results

Patient H was born without complications, 2730 grams at the time of birth. APGAR scores were 9 at 1 min and 9 at 5 min. Head was 32 cm in circumference, and abdominal girth was 28 cm; patient H's weight for length and head size for length were appropriate. There were no neurological abnormalities on the physical exam, and the patient was appropriately responsive to all light, sound, and touch-based stimuli without any evidence of seizures. There were no mucocutaneous lesions, no rashes, no evidence of hepatosplenomegaly. Patient H's vitals were appropriate for age, and the patient was afebrile. Chest X-ray showed no evidence of pneumonia alba or any concern of neonatal respiratory distress syndrome. Clinically, the patient demonstrated no symptoms of congenital syphilis. The only concerning finding was bilateral distal femoral metaphyseal lucency on X-ray of the long bones, but these findings were borderline and contained multiple imaging artifacts. Complete blood count and comprehensive metabolic panel laboratory studies showed no electrolyte imbalance, leukocytosis, or anemia, and head ultrasound showed no hemorrhage or ventricular abnormalities.

Treatment Course

Although the providers had low clinical suspicion, it was decided that this case should be treated like a congenital syphilis case, given the current guidelines. Contact precautions were initiated, and a 10-day course of Penicillin G IV 100,000 u/kg/day required admission to the NICU for administration. The department of public health was also consulted and notified regarding this case. Other laboratory workup was unremarkable for congenital syphilis, including a negative FTA Abs serology study. Ophthalmologic evaluation on the 7th day of life was unremarkable, with no corneal keratitis or chorioretinitis found on examination. The absence of

any other findings besides the possible long bone abnormalities was enough to justify withholding an invasive lumbar puncture procedure, as per neonatologist documentation, unless neurological symptoms or fever developed during care.

Although non-clinical, It is important to mention that when interviewing the nurses attending the patient, they would remark that the mother would often appear distressed at the bedside when visiting the child and vocalized feelings of guilt about potentially infecting her child on multiple occasions. On patient H's 6th day of life, the maternal FTA-Abs repeat assay send out to Quest diagnostics was interpreted as negative, and the department of public health was consulted again. The department of public health advised the providers to complete the course of penicillin as per CDC guidelines. When the provider contacted the mother about the new negative results, she informed them that her husband, with whom she was sexually active, also had a negative FTA-Abs serology study. Patient H's mother expressed a great deal of confusion regarding these results. The clinicians and the department of public health were hesitant to give any definitive answer to patient H's mother concerning how she should interpret the results; instead, focusing on advising her about the need to complete her course of treatment to ensure eradication of any potential infection.

Patient H completed the 10-day IV Penicillin G and was discharged to follow up with an outpatient pediatrician. Mother to Baby H was encouraged to follow up with her PCP or OB for postpartum care and discuss these results in an outpatient setting for further testing. Although discussion of false-positive testing with the mother was planned, she was Spanish speaking, and the times she would visit were sporadic due to issues with arranging transportation. When clinicians wanted to discuss this with her, she could not stay for that discussion due to her need to leave and meet the person who was waiting to transport her home. Due to the delay in arranging professional Spanish translation, a more extensive conference with mom could not be performed. It is uncertain of her level of awareness regarding the potential false-positive result and its implications for future pregnancies.

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Retrospective analysis

Regarding this case, it is essential to discuss the testing and the interventions of this case specifically. The first major topic to bring up is the lapse of prenatal care on the mother's part. Due to this lapse of care, treatment was initiated far too late for even a term baby, as she should have been screened much earlier. Her medical chart mentions that her follow-up with obstetrics was sparse, but it is important to note that insurance was likely not a contributor to poor follow-up, given the fact that all pregnant women qualify for state-supplied medical insurance in this region of the United States. However, a lack of transportation is a significant barrier to care in the US medical system and is responsible for approximately 50% of patient no-shows for appointments(10).

Given that the patient's mother would arrive at unpredictable times and explicitly state that she needed someone else to transport her, it's reasonable to suggest that transportation was likely a factor in failing to meet prenatal guidelines. For this reason, a policy-based solution on behalf of the state insurance organization should be considered. If the system could have lowered the obstacles the patient's mother faced by providing her free transportation, this could have prevented the need to admit patient H altogether. This solution could be more cost-effective for the state insurance organization while also less stressful for the family. This potential solution would need to be further investigated with a cost-benefit analysis by state insurance, but this is something for which to advocate.

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The second feature of this case concerns Patient H's mother's potential false-positive serologic findings. The CDC's data shows the current rate of false-positive findings for FTA Abs immunoassay is 0.26-0.1%, and the false-positive rate of RPR is approximately 3% when screening the general population (8) (11). It is important to note that autoimmune disease can potentially elevate the incidence of false-positive values in both studies, and this patient H's mother is a Hispanic female within a 15- 40-year-old age range, which are all high-risk categories for Lupus (11). Lupus can also cause spontaneous fetal demise in pregnancy (6). However, the patient was not a good historian, and this could not be thoroughly evaluated before delivery, which makes the possibility of Lupus decreasing the positive predictive value of both studies a reasonable explanation. Although the odds of a false positive in both tests are quite low, it is still reasonable and more likely than a false negative serology study in patient H's mother, especially since no evidence of transmission was seen in her husband, with whom she was sexually active. There was an aspect of testing that could have been improved in this case: the sent-out confirmatory test. The test used to confirm was an additional FTA-ABS, sent out to a more skilled laboratory to confirm the diagnosis. Instead, the clinicians should have ordered the more accurate test: the TP-PA, which is more sensitive and specific than FTA Abs and is the current recommended confirmatory test per the CDC (8).

Another area to discuss is clinical decision-making. It is important

to note that before the 6th day when the negative confirmatory test was known, the treating physicians only knew the patient's mother had a positive treponemal and non-treponemal test without appropriate prenatal antibiotic therapy and a history of a past pregnancy ending in fetal demise. This knowledge alone warranted the diagnosis of probable syphilis and treatment as per current CDC guidelines (1). Appropriate care was provided to the neonate promptly. Regarding the decision not to obtain a lumbar puncture, guidelines suggest that this was an appropriate clinical decision. Given the normal neurological exam, initiation of appropriate treatment, and negative serology findings, the lumbar puncture would not have provided any potential benefit. The CDC guidance allows providers to decide against lumbar puncture based on clinical findings and suspicions (1). One might entertain the possibility that X-ray findings of the femoral metaphyseal lucency supported the need for a lumbar puncture. However, studies have shown these X-ray findings are sensitive for congenital syphilitic infection but non-specific and can be a normal anatomical variation seen in the general population (4). This finding is only used to support the clinical decision to initiate treatment, but it is not diagnostic of congenital infection. Even in light of the long bone X-results, withholding lumbar puncture was still clinically appropriate given current guidelines and what is known in the current literature.

The last area of care to discuss is the absence of patient education on discharge about false-positive tests. Communication should be improved, as it could provide patient benefit without any additional cost to the hospital or patient. One important consideration is the stability of a home situation before discharge (1,7). Patients place much faith in the validity of medical testing, and failing to educate the patient about false-positive findings could generate marital animosity and reduce the stability of the nuclear family, which can be attributed to worse neonatal outcomes (11). For this reason, all healthcare sites that test and treat sexually transmitted diseases should consider having a standardized protocol to best educate their patients about false-positive results, to reduce familial tensions and stress prior to discharge. Providing this information could give peace of mind to the entire family while potentially preventing the risk of spousal abuse, divorce, and parental feelings of guilt regarding their child. A system of care should take the responsibility to educate their patients about potential false-positive results, rather than leave it up to the patient's family to figure out which test results should be trusted.

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Discussion

In the modern era of medicine, approximately 70% of all clinical decisions are made through diagnostic testing (2). This practice is seemingly at odds with one of the oldest paradigms taught in medicine: "the test result should confirm what you already know after a thorough history and physical." In this case, many factors stand in opposition to this paradigm, including the fact that this test is a test looking for diseases that clinicians do not suspect, which reduces the positive and negative predictive value. Additionally, the disease being tested is stigmatized in our monogamous society, as it would typically be acquired through extramarital intercourse, creating additional obstacles to taking an accurate medical history. One Web MD survey conducted in 2004 found that 17% of patients will exclude details of their sexual history from their physician when asked (2). In essence, screening for sexually transmitted infections in the general population subverts many aspects of the traditional paradigms surrounding clinical testing, and clinicians should be prepared to think about these test results with greater skepticism. Physicians must remain educated about false positives and false negatives rates within common screening laboratory studies and be ready to question if these are representative of the truth. Admittedly, with congenital syphilis, there is no time to wait for a confirmatory test before therapy, as untreated syphilis can cause widespread irreversible damage in neonates whose immune system is still developing (1). No matter how accurate or expedient the repeat study is, there would likely be no change in the current course of treatment given current CDC guidelines. The intervention of admission and administration of penicillin is relatively harmless, but the harm instead comes from the strife and stigmatization surrounding diagnosis. Therefore, we as medical professionals should give value to those repeat serology findings by calling initial syphilis into doubt in cases such as these to provide reassurance towards the family unit, reduce suspicions of spousal infidelity, ultimately achieve a reduction in neonatal early life instability. These quality improvement suggestions could be a long-lasting solution to improve neonatal quality of life.

Conclusions of this case

1) Insurance companies and other healthcare organizations should consider proactively arranging adequate transportation to an obstetrician for prenatal care if an actively pregnant patient has difficulty obtaining personal transportation. This could potentially improve follow-up rates for prenatal care and may reduce the need to use hospital services for conditions that can be addressed without urgency in the outpatient setting.
2) Providers should order the more sensitive and specific TP-PA assay over TP Abs for confirmational serology of syphilis as per CDC guidelines, whenever patient's insurance can cover it.
3) Hospitals should create a standardized resource that educates patients on the potential of false-positive and false-negative results before screening patients for sexually transmitted infections and give that resource to the patients equitably. This resource should be implemented to reduce stress and tension in the family, providing more holistic care to a neonatal patient focusing on the family unit.

References:

1. *CDC "Congenital Syphilis - Sti Treatment Guidelines." Cen-*

ters for Disease Control and Prevention, Centers for Disease Control and Prevention, 22 July 2021, www.cdc.gov/std/treatment-guidelines/congenital-syphilis.htm.
2. De Noon, Daniel. "Lying in the Doctor's Office: Statistics and Information at Medicinenet.com." MedicineNet, MedicineNet, 10 May 2005, www.medicinenet.com/script/main/art.asp?articlekey=46985.
3. Dickinson, James A. "Screening: When Things Go Wrong." Can Fam Physician, July 2018.
4. Henao-Martinez, A. F., and S. C. Johnson. "Diagnostic Tests for Syphilis: New Tests and New Algorithms." Neurology: Clinical Practice, vol. 4, no. 2, 2013, pp. 114–122., doi:10.1212/01.cpj.0000435752.17621.48.
5. LaFond, Rebecca E., and Sheila A. Lukehart. "Biological Basis for Syphilis." Clinical Microbiology Reviews, vol. 19, no. 1, 2006, pp. 29–49., doi:10.1128/cmr.19.1.29-49.2006.
6. "National Overview - Sexually Transmitted Disease SURVEILLANCE, 2019." Centers for Disease Control and Prevention, 13 Apr. 2021, www.cdc.gov/std/statistics/2019/overview.htm.
7. Peeling, R. "Syphilis." Nature Reviews Disease Primers, vol. 3, no. 1, 2017, doi:10.1038/nrdp.2017.76.
8. Radolf, JD. "Discordant Results from Reverse Sequence Syphilis SCREENING --- Five Laboratories, United STATES, 2006--2010." Centers for Disease Control and Prevention, Centers for Disease Control and Prevention, www.cdc.gov/mmwr/preview/mmwrhtml/mm6005a1.htm.
9. Radolf, Justin D. Medical Microbiology. 4th Edition, "Chapter 36" University of Texas Medical Branch at Galveston.
10. Syed, Samina T., et al. "Traveling towards Disease: Transportation Barriers to Health Care Access." Journal of Community Health, vol. 38, no. 5, 2013, pp. 976–993., doi:10.1007/s10900-013-9681-1.
11 Yen-Lieberman, Belinda, et al. "Identification of False-Positive Syphilis Antibody Results Using a Semiquantitative Algorithm." Clinical and Vaccine Immunology, vol. 18, no. 6, 2011, pp. 1038–1040., doi:10.1128/cvi.05066-11.

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