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## CONGENITAL SYPHILIS CONFIRMED BY PCR AS A RESULT OF TREATMENT FAILURE FOR SYPHILIS IN PREGNANCY. CASE REPORT

**Keywords:** Syphilis, Congenital; Treatment Failure; Penicillin G Benzathine; Syphilis Serodiagnosis; Polymerase Chain Reaction; Nephritis.

**Palabras clave:** Sífilis congénita; Insuficiencia del tratamiento; Penicilina G benzatina; Serodiagnóstico de la sífilis; Reacción en cadena de la polimerasa; Nefritis.

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## RESUMEN

**Introducción.** La sífilis congénita es un importante problema de salud pública y para prevenirla es necesario diagnosticar y tratar la sífilis gestacional de forma temprana. En el presente caso la gestante recibió el tratamiento de elección (penicilina benzatínica), pero este no previno la infección fetal.

**Presentación del caso.** Recién nacido masculino, hijo de una madre con serología negativa para el virus de la inmunodeficiencia humana y positiva para sífilis gestacional diagnosticada en la semana 21 (prueba VDRL con dilución 1:4 y prueba treponémica rápida positiva) y tratada con tres dosis de 2 400 000 UI de penicilina benzatínica. En el parto, la madre presentó VDRL con dilución 1:1 y el recién nacido fue diagnosticado con sífilis congénita por presentar VDRL con dilución 1:4, prueba treponémica rápida positiva, niveles de aspartato aminotransferasa elevados, hipostenuria, proteinuria, hematuria y leucocituria, condiciones que se resolvieron luego de recibir tratamiento con penicilina cristalina durante 10 días. El estudio molecular en sangre realizado al momento del nacimiento evidenció una alta presencia de *Treponema pallidum*. La prueba VDRL a los 3 meses fue no reactiva.

**Conclusiones.** Prevenir la sífilis congénita con el tratamiento recomendado para sífilis gestacional puede fallar, además, diagnosticar sífilis congénita en un recién nacido asintomático es difícil, por lo cual se recomienda hacer un seguimiento clínico y serológico para confirmar si el tratamiento materno fue efectivo en el feto.

## ABSTRACT

**Introduction:** Congenital syphilis is a major public health problem, and early diagnosis and treatment are necessary to prevent it. Penicillin G benzathine is the treatment of choice in pregnant women; however, it may fail to prevent fetal infection, as in the present case.

**Case presentation:** Male newborn, son of an HIV negative mother with gestational syphilis (venereal disease research laboratory (VDRL) 1:4 dilution, positive treponemal test) diagnosed at week 21 of gestation and treated with three doses of 2 400 000 IU of penicillin G benzathine. At delivery, the mother presented VDRL 1:1 dilution. The newborn was diagnosed with congenital syphilis due to VDRL 1:4 dilution, positive treponemal test, elevated aspartate aminotransferases, hypostenuria, proteinuria, hematuria, and leukocyturia that resolved after treatment with crystalline penicillin for 10 days. The molecular testing in blood showed a high treponemal load. The VDRL test at 3 months was non-reactive.

**Conclusions:** Preventing congenital syphilis with the recommended treatment for gestational syphilis may fail. Moreover, diagnosing this condition in an asymptomatic newborn is difficult. Therefore, clinical and serological tests are recommended to confirm whether maternal treatment was effective in the fetus.

## INTRODUCTION

Congenital syphilis (CS) is a serious public health problem that causes about 305 000 perinatal deaths worldwide each year (1).

According to Korenromp *et al.* (2), in 2016, the estimated global prevalence of gestational syphilis (GS) was 0.69% (95%CI: 0.57–0.81), resulting in an overall CS rate of 473 (95%CI: 385–561) cases per 100 000 births. Furthermore, these authors estimated that there were 661 000 (538 000–784 000) cases of CS, with an estimated 355 000 adverse birth outcomes: 143 000 miscarriages and stillbirths, 61 000 neonatal deaths, 41 000 premature and/or low-weight births, and 109 000 clinical cases of CS. In Colombia, 4 270 cases of GS and 777 cases of CS were reported at the cut-off of epidemiological period VII of 2020, showing an increase in 526 (14%) and 124 cases (19%), respectively, compared with the same period in 2019 (3).

About 50% of newborns (NB) with CS are asymptomatic or have subtle and nonspecific manifestations. In addition, more sensitive and specific diagnostic tests, such as enzyme immunoassays, polymerase chain reaction (PCR) test and immunoblotting, are not available in places where the disease is prevalent (4). It has also been shown that cases are not properly investigated due to pressure on health personnel to discharge patients early and the lack of available resources (4).

In their study, Temmerman *et al.* (5) found that women with GS have a higher risk of adverse obstetric outcomes such as stillbirths or low-weight NBs (OR: 4.1; 95%CI: 2.4–7.2); however, these authors also established that prenatal treatment for this condition with reactive rapid plasma reagin significantly improves pregnancy outcomes, although the risk of adverse outcomes remains 2.5 times higher than that observed in uninfected pregnant women.

Early manifestations of CS include maculopapular rash, lymphadenopathy, and hepatic, splenic, hematologic, renal, bone, and central nervous system involvement, but it is important to note that up to 2/3 of infants infected have no manifestations (6). Organ involvement should therefore be investigated using laboratory tests (7).

Penicillin is the antibiotic of choice for treating GS to prevent maternal-fetal transmission and treat CS (8); its effectiveness depends on the stage of the infection in the mother, the number of spirochetes in blood, the severity of fetal infection, the time of initiation of treatment, and the levels of penicillin in fetal tissues (9). Although it has been reported that, in non-pregnant adults, penicillin concentrations as low as 0.018 µg/mL for 7 days result in treponemacidal activity in almost 100% of cases, (10) Nathan *et al.* (11) stated that after administering 2 400 000 IU of benzathine penicillin G (BPG) to 25 full-term healthy pregnant women (38–39 weeks) scheduled for cesarean section the following week, the serum concentration of this drug was <0.018 µg/mL at 7 days in 36% (11).

Appropriate treatment for GS, according to Cerqueira *et al.* (1), includes administration of BPG 30 days or more before delivery and is established depending

on the clinical stage of the disease in the pregnant woman, the availability of documentation confirming the partner's treatment, and the expected drop in VDRL titers. In Colombia, the Clinical Practice Guideline for the Comprehensive Care of Gestational and Congenital Syphilis (12) establishes a treatment that consists of administering 2 400 000 IU of intramuscular BPG in a single dose for pregnant women with early syphilis ( $\leq 1$  year of infection) and 2 400 000 IU of intramuscular BPG at a weekly dose for 3 weeks for late syphilis (latent syphilis  $> 1$  year of duration since infection) and syphilis of unknown duration. These recommendations, according to De Santis *et al.* (13), are the same for patients co-infected with human immunodeficiency virus (HIV).

It should be noted that, despite the existence of published treatment guidelines for GS and CS, Walker *et al.* (14), in a systematic review that included 25 studies (6 146 patients with GS) aimed at determining the optimal treatment regimen (dose, duration, and method of administration) for GS, concluded that while penicillin is effective at treating this infection and preventing CS, there is insufficient evidence to establish the optimal regimen. Additional research is needed to evaluate cases of treatment failure with suggested regimens and the impact of HIV infection in cases of prenatal syphilis treatment failure (14). It should be kept in mind that for years, there has been a belief that the prescribed treatment for GS is ineffective in preventing or treating CS (15,16), as the reported frequency of penicillin failure fluctuates between 2% and 14%. (17)

The following is a case of a NB with renal involvement due to CS as a result of GS treatment failure in the mother. Infection in the NB was confirmed by PCR test for *Treponema pallidum*.

## CASE PRESENTATION

This is the case of a male newborn, the first child of a 30-year-old single Caucasian mother from Bogotá who receives subsidized health care. At week 21 of pregnancy, the woman was diagnosed with GS by VDRL test with dilution 1:4 and rapid positive treponemal test. Additionally, she was tested for HIV, urinalysis, and toxoplasma IgG, all of which were negative, as well as fasting blood glycemia at 2 hours, which was normal, and blood hemoglobin (Hb) at 12.5 g/dL. At that time, the woman reported a history of diagnosis of syphilis 4 years earlier diagnosed through routine occupational examinations, but she received no treatment. Consequently, treatment was initiated with 1 weekly dose of 2 400 000 IU of intramuscular BPG for 3 weeks. The mother also reported that the relationship with the child's father lasted only 3 months, that he was unaware of the pregnancy and that he was the father, and that he had not received treatment for syphilis either. Prenatal ultrasounds, performed at weeks 25 and 34 of gestation, showed no abnormal findings, and the latter reported an estimated fetal weight of 2 430g.

At the time of delivery, the mother underwent a rapid treponemal test that was positive and a VDRL with a dilution of 1:1. The patient was delivered vaginally, at term, with normal amniotic fluid and cord clamping; his weight was 3 000g; his height was 49cm; and his head and chest circumferences were 35cm and 32cm, respectively. His APGAR scores at 1 minute, 5 minutes, and 10 minutes were 8, 9, and 9, respectively. The placenta was completely removed and showed no signs of infection.

On physical examination at birth, the only finding of relevance was sutural diastasis with wide anterior fontanelle. At 13 hours of life, the patient underwent a blood typing test, which was A+, and a VDRL test with a dilution titer of 1:4. Therefore, the patient was hospitalized, and treatment was started with 150 000 IU of intravenous crystalline penicillin every 12 hours. On the same day, blood count, long bone X-ray and transfontanellar ultrasound were performed, which were normal, and tests for aspartate aminotransferase, alanine aminotransferase, indirect bilirubin and conjugated bilirubin yielded the following results: 57 IU, 13 IU, 7.1 mg/dL, and 0 mg/dL, respectively.

Urinalysis showed pH of 6.5, density of 1 005, proteins at 25, Hb of 150, and leukocyte esterase of 100; nitrite, glucose and ketone tests were negative and urobilinogen was normal. Sediment analysis revealed the following results: epithelial cells: 5-10 HPF, transitional cells: 2-5 HPF, superficial layer of renal epithelial cells: 0-2 HPF, leukocytes: 10-15 HPF, red blood cells: 10-15 HPF, amorphous urate crystals: +++, ammonium biurate crystals: +++; urea nitrogen and creatinine levels were normal. Additionally, a lumbar puncture was performed to rule out neurosyphilis, as well as a rapid treponemal test, which was positive.

Based on the findings, the patient was diagnosed with GS with renal and hepatic involvement.

At eight days of birth, and after receiving treatment, the patient's crystalline penicillin dose interval was switched to every 8 hours according to the protocol; a follow-up analysis carried out the following day showed a significant improvement, which supported the diagnosis of CS with renal involvement. After 10 days of in-hospital treatment with crystalline penicillin, he was discharged and outpatient treatment was indicated.

To establish the molecular diagnosis, the remnant of the serum and whole blood samples taken from the NB were used; DNA was extracted from these samples and the *TpN47* gene was detected. The blood sample tested was considered positive with a threshold cycle of 19.87; this result showed a high presence of initial *T. pallidum*, reaching an exponential amplification of  $5.73 \times 10^6$  copies at the end of the reaction.

The follow-up VDRL test performed at 3 months of life was non-reactive.

## DISCUSSION

### Diagnosing the newborn

CS is diagnosed based on the epidemiological link to the infected mother who has not been treated or has received insufficient treatment, and/or on laboratory and physical examination results in an NB born to a mother who has a history of syphilis.

According to the CDC's 2015 Sexually Transmitted Infections Treatment Guidelines (18), a confirmed or highly probable case of CS is considered when the physical examination of an NB shows findings consistent with this condition, the antibody titer blood test is four times higher than the maternal titer, or treponema is identified via dark-field microscopy or PCR test in lesions or body fluids. However, as indicated by Cooper & Sanchez (19), NBs with CS may have lower titers than their mothers.

Routine PCR and multiplex PCR techniques are used to diagnose CS in the early stages of infection, when the serological reaction is negative, whereas nested PCR and real-time PCR are more appropriate for confirmation (20). In addition, several PCR variants have been used to identify different molecular targets, such as the genes *TPF-1*, *16S rDNA*, *Pola*, *tpp47*, *bmp*, *TMPA*, *tppB*, and *TpN47*. The additional clinical value of the tr-TaqMan PCR assay targeting the *polA* gene of *T. pallidum* in diagnosing syphilis using various algorithms is also highlighted (21).

Although the *TpN47* gene has the highest sensitivity and specificity in the diagnosis of CS, molecular typing could be performed using the *16S ADNr* and *polA* genes. Therefore, it becomes an important tool for monitoring the emergence of macrolide-resistant strains, to assess disease subtypes associated with central nervous system disease, to differentiate infection and reinfection processes, and to understand *T. pallidum* transmission and the epidemiological behavior of the disease; the *arp* (acidic repeat protein) gene and the subfamily II *tpr* genes (*tprE*, *tprG*, and *tprJ*) have been used for the latter purpose (22). Furthermore, the *rpsA* and *tpo548* genes are being studied together to improve the discriminatory ability of *T. pallidum* strain typing (23). It should be noted that there is currently no commercial PCR-based test for the diagnosis of syphilis.

In the case reported, the NB met the following diagnostic criteria for CS: VDRL test 4 times the maternal titer and laboratory tests suggestive of CS due to increased aspartate aminotransferase and urinalysis alterations; in addition, *T. pallidum* was identified based on the detection of the *TpN47* gene using a PCR test, which has been shown to be a more sensitive and specific test.

### Transplacental infection and failure of maternal treatment to prevent CS

Transplacental transmission of *T. pallidum* to the fetus is related to gestational age, stage of infection, and fetal immune response (13). This may occur as early as week 9 or 10 of pregnancy, although the majority of infections occur during the second

trimester (19). Likewise, the risk of fetal infection is higher during the early stages of GS, possibly due to rapid replication of the microorganism and the increased concentration of spirochetes in the bloodstream; thus, a higher risk of transmission has been reported in cases of untreated primary and secondary syphilis (70% to 100%) compared with untreated latent syphilis (40% for the early stage and up to 10% for the late latent stage) (24). In the case reported here, the stage of the infection in the mother, who was diagnosed in the second trimester of pregnancy, was not known.

Based on a study in which 75 women with GS were treated with BPG and treatment failure was demonstrated in 4 of them, resulting in a failure rate of 5.3%, Monif (15) warned of the need for a critical reassessment of the established treatment for syphilis in pregnant women with a high probability of established fetal infection.

Regarding the management of syphilis in pregnant women, Alexander *et al.* (25), through a prospective evaluation of the recommended treatment regimens for syphilis between 1987 and 1989 at Parkland Memorial Hospital in Dallas, Texas, found that 28 552 women gave birth in the institution and 448 of them were diagnosed with the disease; 108 of these women were diagnosed at the time of delivery and treated after delivery, while the remaining 340 received prenatal care. In the latter group, 6 cases of failure in the prevention of congenital syphilis were found, 4 of them in women with secondary syphilis and 2 in early latent syphilis (failure 1.76%).

In a prospective study conducted between January 31, 1982, and December 31, 1998, including all women who received prenatal care at hospitals in Dallas, United States, Sheffield *et al.* (10) identified 43 cases of CS. The authors established that, in all these cases, pregnant women received treatment after the first trimester, with the mean interval from treatment to delivery being  $13.5 \pm 5.4$  days, and 15 women (35%) were treated 30 days or more before delivery (32-183 days). In turn, Rac *et al.* (26), in a retrospective study that included 235 pregnant women with syphilis diagnosed after week 18, found that of the 73 (30%) who had an ultrasound diagnosis of fetal syphilis and were treated before delivery, 32 (18%) gave birth to NBs with CS.

The majority of failed CS treatments appear to occur in cases of secondary syphilis (10,25); when the duration of infection in the pregnant woman is <1 year (27), possibly due to the high number of spirochetes in the blood that occur at this stage (24); or when the mother is diagnosed or treated in the third trimester (28-31). In a systematic review that included 25 observational studies, Blencowe *et al.* (31) found that diagnosing and treating GS after weeks 24 to 28 of gestation is a risk factor for CS, stillbirths, preterm delivery, and neonatal death.

Transplacental transmission of *T. pallidum* is also difficult to prevent when the duration of syphilis in the mother is unknown (32); when the titer of VDRL or RPR (rapid plasma reagin) is very high at diagnosis and during delivery (10,28,29,32); when the time between treatment and delivery is short; when

delivery occurs before week 36 (10,33); and when CS manifestations are detected on antenatal ultrasounds (26,34,35), which are recommended by the CDC in the second half of pregnancy if GS is diagnosed (36), with the most frequent findings being hepatomegaly, ascites, hydrops fetalis, fetal anemia, polyhydramnios, and placentomegaly (27,34).

Donders *et al.* (37) conducted a study of 180 HIV-negative pregnant women diagnosed with syphilis from Petronas, South Africa, in which 108 received 2 or 3 injections of 2 400 000 IU of BPG weekly and had favorable results; however, there was an increase in adverse outcomes in the group that received a single injection. When the authors compared the estimated duration of treponemicidal coverage of 3 weeks or less with that of more than 3 weeks, a decrease in birth weight was observed (2 748g vs. 3 130g); in addition, the relative risks of prematurity (RR: 8.5; 95%CI 2.5-28), perinatal mortality (RR: 20.5 95%CI 2.3-184) and congenital syphilis (RR: 2.0 95%CI: 0.6-6.8) increased when coverage was less than 3 weeks, suggesting that the duration of treatment is more important than the time of the first injection. Therefore, 2 400 000 IU of BPG or treponemicidal concentrations lasting 3 weeks or less is not sufficient to treat GS and prevent CS (37).

Antibiotic treatments other than penicillin (38-40) and reinfection may facilitate transplacental transmission of syphilis. Furthermore, since serology is used to evaluate active infection and requires long-term follow-up to assess the effectiveness of treatment, treatment failures may be undiagnosed reinfections (32). These failures may occur in cases of maternal neurosyphilis, as BPG is not effective for its treatment (41).

In the present case, the diagnosis of GS was made at the beginning of the second half of pregnancy; the VDRL titer was not high (dilution 1:4) and decreased at delivery (dilution 1:1); the stage of infection in the mother was not known and she was treated with 3 doses of 2 400 000 U of BPG weekly; 14 weeks elapsed between the start of treatment and delivery; ultrasounds performed at weeks 25 and 34 were normal; and the mother was HIV-negative and had not received any antibiotics other than penicillin.

Therefore, the only risk for transplacental transmission of the disease would be the unknown duration of infection; however, the decrease in VDRL titers from 4 dilutions to 1 dilution in 3 months met the expected decline for early syphilis (42) and the retention of the same titer at the time of delivery ruled out reinfection according to the Evidence-based Clinical Practice Guideline for the Comprehensive Care of Gestational and Congenital Syphilis. (12) Consequently, it can be concluded that the treatment was effective for the mother but not for the fetus. This is significant because it is assumed that compliance with the criteria for appropriate treatment in a pregnant woman ensures prevention of infection or treatment of the infected fetus (26).

So, what happened in this case to make the mother's treatment ineffective for preventing CS? Since the presence of *T. pallidum* was identified via PCR test

and the VDRL titer in the NB was 4 times higher than the maternal titer (which is evidence of fetal serological response), penicillin levels may not be sufficient to eradicate the bacteria in the fetus. Several factors may contribute to this outcome; for example, if the number of spirochetes in the mother's blood is high, a placental alteration may occur which, together with increased renal flow at the end of pregnancy, may result in decreased penicillin levels (9). In addition, the increase in plasma volume and renal clearance in pregnant women can reduce serum levels between 10% and 50% (43,44).

### Renal involvement in CS

Renal involvement is not a common complication in cases of CS. In a study carried out in 28 NBs with CS treated between August 2011 and February 2012 at the Instituto Materno Infantil of Bogotá, Vallejo & Cifuentes (7) found that only 17.9% of the participants had some renal alterations.

It has been established that when renal alterations occur in cases of GS, the most common involvement is a nephrotic syndrome as an isolated manifestation (45), which is quickly resolved with penicillin treatment, but it can also manifest as a systemic involvement (46) or with proteinuria, hematuria, leukocyturia, and cylindruria (7). Renal involvement, a serological reaction, the presence of *T. pallidum*, and a diagnosis of GS were all observed in the case presented. In addition, alterations were resolved using the penicillin treatment introduced by Scully and Yamazaki in 1949 (47).

## CONCLUSIONS

Syphilis is a complex disease and its treatment during pregnancy must meet several objectives: to eradicate the infection in the mother, to prevent maternal-fetal transmission, and/or to treat CS; therefore, situations involving the risk of maternal treatment failure must be investigated. Thus, given that the diagnosis of CS depends on the diagnosis of the pregnant woman, these risk situations for maternal treatment failure should be considered when treating a NB with a maternal history of treated GS.

In this regard, since preventing CS with the recommended treatment for GS may fail and because diagnosing CS in an asymptomatic NB is difficult, clinical and serological follow-up is recommended to confirm whether maternal treatment was effective for the fetus.

## ETHICAL CONSIDERATIONS

The patient's mother provided an informed consent that allowed preparing this case report.

## CONFLICT OF INTEREST

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To the patients, who always teach us.

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