New perspectives in the study of the congenital syphilis: A narrative review

Novas perspectivas no estudo da sífilis congênita: Uma revisão narrativa

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ABSTRACT
Syphilis is still an infection of public health significance, particularly due to its impact during pregnancy and the possibility of mother-to-child transmission that may occur at any stage of pregnancy. Congenital syphilis can lead to miscarriage, stillbirth, perinatal death, premature birth, and clinical manifestations in the newborn. In prospective, the perinatal morbidity and mortality rates due to congenital syphilis are even greater than those due to HIV infection. Overall, both syphilis and congenital syphilis remain a serious public health issue mainly due to flaws both prenatal care systems and syphilis prevention and control programs. This review discusses vertical transmission of the syphilis agent the Treponema pallidum subsp. pallidum, the clinical manifestations of congenital syphilis, the current guidelines for the evaluation and treatment of infants born to mothers with gestational syphilis, the global epidemiology and ongoing research efforts to better understand the pathogenetic mechanisms employed by the syphilis agent to cross the placental barrier and cause congenital infection.

Keywords: Congenital syphilis, gestational syphilis, Treponema pallidum.

RESUMO
A sífilis ainda é uma infecção de importância para a saúde pública, principalmente devido ao seu impacto durante a gravidez e à possibilidade de transmissão de mãe para filho que pode ocorrer em qualquer estágio da gravidez. A sífilis congênita pode levar a aborto espontâneo, natimorto, morte perinatal, parto prematuro e manifestações clínicas no recém-nascido. Em prospectiva, as taxas de morbidade e mortalidade perinatal por sífilis congênita são ainda maiores do que aquelas por infecção por HIV. No geral, a sífilis e a sífilis congênita continuam sendo um grave problema de saúde pública, principalmente devido a falhas nos sistemas de assistência pré-natal e nos programas de prevenção e controle da sífilis. Esta revisão discute a transmissão vertical do agente da sífilis, o Treponema pallidum subsp. pallidum, manifestações clínicas da sífilis congênita, diretrizes atuais para avaliação e tratamento de bebês nascidos de mães com sífilis gestacional, epidemiologia global e esforços de pesquisa em andamento para entender melhor os mecanismos patogenéticos empregados pelo agente da sífilis para atravessar a barreira placentária e causar infecção congênita.

Palavras-chave: sífilis congênita, sífilis gestacional, Treponema pallidum.
1 EPIDEMIOLOGY

The current global epidemiology of gestational and congenital syphilis reveals the failure of the control measures aiming at their prevention. In 2017, of 83 countries notified, 37 reported more than 1% of pregnant women diagnosed with syphilis (1) (2). Congenital Syphilis is the second leading cause of fetal loss and it is estimated that 1.4 million pregnant women are infected with syphilis every year and 520,000 adverse outcomes are estimated. Such outcomes include 215,000 deaths, 90,000 neonatal deaths, 65,000 premature or low birth weight babies and 150,000 infants with congenital disease (2) (3).

An alarming resurgence of syphilis has been observed in the United States, due to higher incidences of primary and secondary syphilis among women in childbearing age. The United States reported that 918 babies were born with syphilis in the USA in 2017. The number has risen from 362 in 2013 and reached a 20-year high after years of sustained reduction. Five states, Florida, California, Arizona, Texas, and Louisiana—accounted for 70% of the cases. Florida, California, and Texas have some of the highest immigration levels in the USA and Louisiana is one of the poorest states (4) (5). In 2017, 36 confirmed cases of congenital syphilis were reported in 10 European Union (EU) countries. Poland reported one confirmed case and eight cases with unknown classification. Thirteen countries reported no cases. Only Bulgaria and Romania reported more than five cases in 2017 (14 and 6 respectively). The total number of reported congenital syphilis cases remained stable in 2017 compared with 2016, when 37 cases were reported. The crude rate of reported congenital syphilis in the EU was 1.1 cases per 100 000 live births and has remained stable since 2015 (6) (7) (8). The estimated prevalence and incidence of syphilis varied substantially by region or country, with the highest prevalence in Africa and >60% of new cases occurring in low- and middle-income countries (LMICs). The greatest burden of maternal syphilis occurs in Africa, representing >60% of the global estimate (9).

One consequence of the infection caused by Treponema during pregnancy is the predisposition to the HIV virus. Syphilis, similar to other sexually transmitted infections (STIs), acts synergistically with HIV. The risk of infection with the HIV virus is increased in patients with syphilitic ulcers because of the disruption of the epithelial barriers and due to the presence of T CD4 lymphocytes in the ulcers (10). When syphilis is asymptomatic or undiagnosed, it favors negative prognoses in HIV patients since it reduces CD4 cell counts and increases the viral load (11).

For populations in developing countries, the global burden of mother-to-child transmission (MTCT) of HIV and Syphilis continues to reflect the limited access to quality prenatal care services. As a result, several strategies at the regional and global levels have been put forward to eliminate
these two diseases (12). Countries such as Cuba, Thailand, Belarus, and four United Kingdom overseas territories have reported the successful elimination of both MTCI of HIV and syphilis. Similarly, Moldova reported having achieved the elimination of congenital syphilis; while Armenia reported the elimination of MTCI of HIV. It is worth noting that while there has been significant progress in Asia and the Americas, the prevalence rates of maternal syphilis and HIV have, to a considerable extent, remained stagnant in Africa (9).

2 GESTATIONAL AND FETAL INFECTION

The syphilis causative agent, *Treponema pallidum* subsp, *pallidum (T. pallidum)* infect a new host mainly through sexual contact with an infected individual carrying an active early lesion, normally rich on treponemal cells. Vaginal, anal, or oral sex are all practices associated to syphilis transmission, but infection might also occur when the pathogen reaches dermal microabrasions on the skin (9) (13).

In pregnant women, gestational syphilis (GS) can be associated with transplacental transmission of the syphilis agent, which can occur at any time during gestational syphilis but it happens with increasing frequency during the second half of the gestational period (14) (15). Transmission of syphilis to the fetus is directly related to the stage of the infection. Women with untreated primary or secondary syphilis are more likely (60-90% probability) to transmitted infection to their fetuses than women with latent infection (40% probability in early latent and <10% probability in late latent syphilis). The risk of transmission decreases with increasing time since active infection, and is only ~2% after four years since early manifestations occurred (16) (17). Congenital syphilis is caused by *T. pallidum* invasión of the fetus bloodstream, which, in turn, leads to dissemination to virtually all organs. Clinical manifestations of congenital syphilis are influenced by gestational age, stage of maternal syphilis, maternal treatment, and immunological response of the fetus. Pregnancies complicated by syphilis may result in intra-uterine growth restriction (IUGR), non-immune hydrops fetalis (NIFH), stillbirth, preterm delivery and spontaneous abortion, with the latter generally occurring after the first trimester (18) (19).

The clinical manifestations result from the inflammatory response driven by the pathogen, and their severity can range from radiographic abnormalities to multi-organ system failure, any organ system can be affected. In absence of treatment, active infection can manifest in the fetus, the newborn, or later during child development (20). The clinical manifestations of congenital syphilis (CS) are arbitrarily defined as “early” when they are diagnosed during the first two years of age (including fetal death), and “late” if their onset occurs after two years of age. Approximately 60% of
neonates born with congenital syphilis are asymptomatic at birth (21). Those that are however symptomatic are commonly affected by hepatomegaly, jaundice, nasal discharge (snuffles), generalized lymphadenopathy, anemia, condyloma lata, and petechiae, common skeletal abnormalities include osteochondritis and periostitis. Neurological abnormalities include aseptic meningitis and chorioretinitis (see Table 1). Early CS usually manifests in untreated infants born without symptoms by three months of age, but most often by five weeks (18). Late CS is defined by clinical manifestations whose onset occurs after two years of age. Such manifestations are related to scarring or persistent inflammation from the early infection and are characterized by gummatous lesions in a variety of tissues and organs (22). Late CS develops in about 40% of infants born to women that contracted syphilis during pregnancy but were not treated. Some of the late CS symptoms can be prevented or revert if a woman with syphilis is treated during pregnancy or the newborn is treated within the first three months of life (23). However, other manifestations (eg, keratitis, saber shins) may occur even when appropriate treatment is administered (24).

Table 1. Manifestations and sequels of congenital syphilis.

| Clinic Manifestation | Early Congenital Syphilis | Late Congenital Syphilis |
|----------------------|---------------------------|-------------------------|
| Facial features       | Frontal bossing (Olympian brow), saddle nose, short maxilla, protuberant mandible. |
| Megalias             | Hepatomegaly occurs in up to 50% of cases, splenomegaly, and lymphadenopathy. | x |
| Hematologic manifestations | Anemia, lymphocytosis, monocytes, leukopenia or leukemoid reaction, trombocytopenia with petechiae and purpura occur in approximately 30% of patients; hemolytic anemia Coombs-negative, polychromasia, erythroblastemia diffuse intravascular coagulation, hemophagocytosis, hydrops fetalis. | x |
| Rhinitis             | It occurs between the first week and the third month of life. It is characterized by mucous discharge (high treponemal load) that becomes progressively more profuse and occasionally bloody. | It may lead to destruction of the nasal cartilage which is known as saddle nose. |
| Mucocutaneous manifestations | Jaundice, maculopapular rash, symmetrical superficial desquamation skin especially over the soles and palms. Another form of skin lesion is syphilitic pemphigus and condylomas that occur in | Linear scars that become fissured or ulcerated are not often and they are called rhagades. |
### Bone Injuries

Osteitis, periostitis and osteochondritis occur in 80 to 90% of cases. Cortical demineralization in the metaphyseal and diaphyseal portions of long bones and osteochondritis, which affects the joints, primarily knees, ankles, wrists, and elbows. Osteitis alternating linear bands of translucency and radiodensity of long bones. Parrot’s pseudoparalysis. Little fingers of hands short (Dubois sign), saber shin, the thickening of the sternoclavicular joint (Higoumenakis’ sign) and Clutton’s joints characterized by synovitis with hydrarthrosis. Perforation of the hard palate (virtually pathognomonic for congenital syphilis).

### Renal manifestation

Nephrotic Syndrome that usually occurs between 2 to 3 months of life, the predominant manifestation being generalized edema, including pretibial, scrotal, and periorbital areas, together with ascites. Azotemia, Hematuria, proteinuria, interstitial syphilitic nephritis.

### Ocular manifestations

Salt and Pepper Chorioretinitis, glaucoma, uveitis, cataracts. Interstitial keratitis (bilateral, usually occurs around puberty, but can occur anytime between 4 and 30 years), chorioretinitis, secondary glaucoma, corneal scarring, optic atrophy.

### Auditory alterations

Sensorineural hearing loss associated with late congenital syphilis typically develops suddenly at 8 to 10 years of age and often accompanies interstitial keratitis. The higher frequencies are affected first; normal conversational tones are affected later. Syphilis-associated hearing loss may respond to long-term glucocorticoid therapy.
### Dental Involvement

Hutchinson's teeth abnormalities of the maxillary central incisors), mulberry molars (maldevelopment of the cusps of the first molars).

### CNS Involvement

| Elevated cell count or protein in cerebrospinal fluid, mild hydrocephalus, cranial nerve palsy. Meningitis, meningeal irritation, bulging fontanelles and abnormal pituitary function. | Mental retardation, hydrocephalus, seizures, cranial nerve abnormalities including blindness and deafness, juvenile general paresis, cranial nerve palsies. |

### Others

| Placental villitis or vasculitis (unexplained enlarged placenta), intrauterine growth restriction, preterm birth, alopecia, fever, pneumonia alba, fibrosing pneumonitis, myocarditis, pancreatitis, rectal bleeding owing to syphilitic ileitis, necrotizing enterocolitis, malabsorption secondary to fibrosis of the gastrointestinal tract, and fetal bowel dilation. Diarrhea caused by malabsorption due to inflammation and fibrosis in the genital tract. | x |

Source: elaborated by the authors based on (6) (23) (66).

Early congenital syphilis is suspected when serological tests routinely performed during pregnancy have reactive and/or positive results in pregnant women. Newborns of mothers with this serological evidence should undergo a thorough analysis that includes dark-field microscopy or immunofluorescent staining of skin, mucous membranes, placenta and umbilical cord if possible, in addition to a quantitative non-treponemal serologic test (e.g. VDRL) (23) (25). Infants with clinical signs of disease or suggestive results of serological tests should have a CSF analysis for cell, VDRL and protein counts; a complete blood count with platelet count; liver function tests; X-ray of long bones; and other ophthalmological evaluation tests, chest X-rays, neuroimaging tests and auditory brainstem response (ABR) (6) (18) (26).

The diagnosis is confirmed by means of microscopic visualization of spirochetes in samples of the infant or placenta. Diagnosis based on neonatal serological tests becomes more complicated due to the transplacental transfer of maternal IgG antibodies. However, an infant’s non-treponemal antibody titer fourfold higher than that of the mother is deemed as confirmed or highly probable (27) (28). The diagnosis of late CS is based on the medical records, distinctive physical signs and positive
serological tests (see Table 2) (29). Hutchinson’s triad: interstitial keratitis, Hutchinson's teeth and deafness of the eighth cranial nerve is diagnostic (20). Sometimes, non-treponemal serological tests are negative, but the fluorescent treponemal antibody-absorption (FTA-ABS) test is positive. The diagnosis should be considered in cases of unexplained deafness, progressive intellectual deterioration or keratitis (30) (31) (32).

Table 2. Case definitions of congenital syphilis

| Case Classification | Description |
|---------------------|-------------|
| Confirmed           | A case that is laboratory confirmed by Demonstration of Treponema pallidum by: • Darkfield microscopy of lesions, body fluids, or neonatal nasal discharge, or • Polymerase chain reaction (PCR) or other equivalent direct molecular methods of lesions, neonatal nasal discharge, placenta, umbilical cord, or autopsy material, or • Immunohistochemistry (IHC), or special stains (e.g., silver staining) of specimens from lesions, placenta, umbilical cord, or autopsy material. |
| Probable            | A condition affecting an infant whose mother had untreated or inadequately treated* syphilis at delivery, regardless of signs in the infant, or an infant or child who has a reactive non-treponemal test for syphilis (Venereal Disease Research Laboratory (VDRL), rapid plasma reagin (RPR), or equivalent serologic methods) AND any one of the following: • Any evidence of congenital syphilis on physical examination • Any evidence of congenital syphilis on radiographs of long bones • A reactive cerebrospinal fluid (CSF) venereal disease research laboratory test (VDRL) test |
In a nontraumatic lumbar puncture, an elevated CSF leukocyte (white blood cell, WBC) count or protein (without other cause):
Suggested parameters for abnormal CSF WBC and protein values:
During the first 30 days of life, a CSF WBC count of >15 WBC/mm³ or a CSF protein >120 mg/dL.
After the first 30 days of life, a CSF WBC count of >5 WBC/mm³ or a CSF protein >40 mg/dL, regardless of CSF serology.

Source: elaborated by the authors based on (28).

3 ETIOLOGIC AGENT

*T. pallidum* is a spirochete bacterium belonging to the *Spirochaetaceae* family. It is a spiral-shaped, microaerophilic bacterium of approximately 5 to 10 μm in length and 0.1 to 0.23 μm in diameter (33) (34). Is the structure of this obligate pathogen is similar to that of a gram-negative bacterium. The as the cytoplasmic membrane is enclosed by a loosely associated outer membrane. A thin layer of cell Wall between the membranes provides structural stability (13) (35). The flagella, responsible for the characteristic corkscrew motility of *T. pallidum*, are located in the periplasmic space, and therefore referred to as endoflagella (36). Because of its double-membrane structure, *T. pallidum* is conveniently described as a Gram-negative pathogen. This analogy is however not accurate both biochemically and ultrastructurally *T. pallidum* outer membrane in fact is devoid of lipopolysaccharide and its phospholipid composition differs from that of more conventional Gram-negative bacteria (9) (36). Although *T. pallidum* most abundantly expressed proteins are lipoproteins, these molecules generally reside in the periplasmic space rather than being surface-exposed. Accordingly, this paucity of surface-exposed antigens diminishes the ability of this spirochete to trigger the host innate immunity during infection, facilitating replication at the site of infection and dissemination to distant sites (13) (36) (37).

In spite of being predominantly periplasmic, convincing evidence has now surfaced that the syphilis spirochete displays very limited amounts of lipoproteins on its surface that appear to be important virulence factors (13) (35). For example, TP0751 (or pallilysin) is a laminin-binding lipoprotein and zinc-dependent metalloproteinase with the ability to degrade extracellular matrix
components. Although poorly expressed by *T. pallidum*, Tp0751 surface-exposure was demonstrated by knock-in experiments in *Borrelia burgdorferi* and *T. phagedenis*, and opsonophagocytosis assays in *T. pallidum* (38) (39) (40). Additionally, the lipoprotein Tpp17 (encoded by the tp0435 gene) has been shown to be at least partially surface-exposed and can function as a placental adhesin (41) (42).

*T. pallidum* limited surface antigenicity fosters evasion of adaptive immune responses hence facilitating persistence. The fragile nature of their outer membrane, makes extremely difficult the identification of *T. pallidum* repertoire of surface-exposed proteins (13) (36) (43). The *T. pallidum* repeat (Tpr) proteins, a 12-membered family of paralogs with homology to the major outer sheath protein (Msp) of the oral pathogen *T. denticola*, are among the most studied putative outer membrane proteins of the syphilis agent. Of these, TprK (TP0897) has been shown to have a role in immune evasion by the spirochete, due to the it’s ability to undergo antigenic variation in seven regions predicted to be extracellular loops. DNA donor cassettes in the *T. pallidum* chromosome have been proposed contain the genetic information that recombines into the tprK variable regions by unidirectional gene conversion (13) (36) (44). Recombinant TprC and TprI have been shown to form trimeric β-barrels when refolded in vitro, induce an increase in permeability when inserted into liposomes, and to be targets of opsonic antibodies. Such evidence supports that these Tprs are function as porins, to import nutrients into the periplasmic space from the host milieu (44) (45).

The analysis of the *T. pallidum* genome confirmed what had been hypothesized based on previous experimental observation, namely that *T. pallidum* has a striking lack of metabolic capabilities. The pathogen can carry out glycolysis but lacks Kreb’s cycle enzymes and oxidative phosphorylation chain (33) (34). Absence of pathways for the use of alternative carbon sources for energy production, as well as for synthesis of enzyme cofactors, amino acids, fatty acids and nucleotides is also suggested by genome analysis, even though *T. pallidum* genome encodes enzymes for the interconversion of amino acids and fatty acids (13) (46). Due to this lack of metabolic and catabolic pathways, *T. pallidum* must obtain essential macromolecules from the host, which explains the obligate nature of this pathogen and its limited ability to survive outside of a susceptible host as well as the very long generation time of this pathogen (30 to 33 hours) (13) (44).

*T. pallidum* extreme adaptation to a host for survival is also testified by its high sensitivity to host-and environment-induced stresses, such as the limited ability of the pathogen to counteract the effects of reactive oxygen species (ROS), due to the absence of genes coding for catalase, oxidase, superoxide dismutase, and glutathione peroxidase (44) (47). Limited protection from environmental stresses is possibly provided by the Tp0092 gene, predicted to code for the pathogen's only annotated extra cytoplasmic function σ factor, homologous to RpoE, and known in Escherichia coli to control
a key transduction pathway for maintenance of envelope homeostasis in response to external stress and cell growth (33) (34) (44). The metabolic inability of the spirochete is compensated by a large number of predicted or experimentally identified membrane transporters with different substrate specificity. For example, the lipoprotein TpN32 (encoded by the Tp0821 gene) was identified as a methionine-binding protein that is likely part of a methionine transport system in *T. pallidum*. Six additional *T. pallidum* transporters have specificity for cations, such as the ATP-binding cassette (ABC) transporter encoded by the Tro operon (Tp0163-Tp0167) (13) (35) (48). TroA, the cation-binding protein of the Tro complex, binds zinc and manganese. *T. pallidum* homologs to dct and y40 encode proteins that are likely to transport sugar molecules across the cytoplasmic membrane. In gram-negative bacteria, the ABC transporter MglABC has specificity for galactose. The homologous Mgl system in *T. pallidum* may also bind galactose. However, due to its inability to utilize galactose as a carbon source, *T. pallidum* may utilize MglABC as a glucose transporter. Ribose may be uptaken via a different ABC transporter homologous to the RbsAC transporter of the spirochete *Borrelia burgdorferi*. However, because ribose is not degraded by *T. pallidum*, also the RbsAC system may function to transport other sugars in *T. pallidum* (44) (49).

**4 GENOME AND MOLECULAR ARCHITECTURE**

The first genome of *T. pallidum* subsp. *pallidum* (strain Nichols) was sequenced in 1998. This pathogen has a comparatively small genome (1.138Mbp) and only ~55% of *T. pallidum*’s 1041 annotated open reading frames are recognized to have a biological function (50) (51). Currently there are approximately 67 sequenced genomes belonging to different *T. pallidum* strains obtained from patient sample or isolated using the rabbit model of syphilis. All genomes are virtually isogenic, sharing a level of identity that is above 99% (52). Arora et al., examined the genomic diversity of such strains collected over the 20th and 21st centuries, and concluded that all *T. pallidum* samples available to date shared a common ancestor that was circulating at the beginning of the 16th century (53). Furthermore, their analysis confirmed a modern epidemic cluster displaying the population genetic and epidemiological features of an emergent pandemic. Such cluster (or clade) was named SS14-Ω based on the fact that the genomes most modern strain analyzed clustered with the SS14 strain genome of *T. pallidum*, isolated in 1977 but sequenced in 2008, while only few genomes clustered with that of the Nichols strain, which was isolated in 1912 and is the main laboratory strain for syphilis research (2) (52).
5 HOST IMMUNE RESPONSE

The scarcity of surface-exposed antigens in *T. pallidum* outer membrane facilitates local replication of the pathogen and dissemination. The increase in the antigenic mass of the pathogen, however, eventually triggers immune recognition. As a result, pathogen cells are uptaken up by dendritic cells, which then traffic to lymph nodes to present treponemal antigens to naive B cells and T cells. The production of antibodies directed against surface antigens induces the uptake and clearance of *T. pallidum* by opsonophagocytes (9) (54). During this process, released lipopeptides bind to Toll-like receptors contained in the phagosome, while other antigenic peptides can be presented to T cells (Figure 1). In turn, at the site of infection, activated T cells produce IFN-γ, which enhances phagocytosis and other cytokines, such as tumor necrosis factor (TNF) and IL-6. Immunohistochemical studies of early lesions have highlighted the presence of CD4+ and CD8+ T cells, natural killer cells and activated macrophages. Perivascular infiltration of lymphocytes, histiocytes and plasma cells is also a common finding along with the swelling and proliferation of endothelial cells (55) (56).

Figure 1. Immune response to *Treponema pallidum*.

Description: The uptake and elimination of *T. pallidum* by the macrophages and the antigenic presentation to T cells occurs simultaneously. Activated T cells produce IFN-γ as well as other proinflammatory cytokines and chemokines which promote the activation of macrophages, cytotoxic T cells, and natural killer cells in the site of infection. Additionally, Th2 profile response is also activated inducing the production of specific antibodies (Opsonins). Created with Biorender.com. Source: elaborated by the authors.
T. pallidum is an extracellular pathogen that can replicate, disseminate, and persist in spite of a robust immune response since the early stages of the infection. Phagocytosis assays have demonstrated that during infection a sub-population of treponemes resistant to immune clearance eventually emerges (57). Such subpopulation of cells that are not recognized by host antibodies is likely directly responsible for pathogen persistence, even though the molecular basis of this phenomenon are largely unknown (9) (58). Understanding the mechanisms that alter T. pallidum surface antigenicity is critical to explain this ability to avoid opsonization and clearance. There are likely several factors contributing to this phenomenon, which include limited availability of the surface target, production of antibodies directed to immunodominant antigens that are however not surface-exposed, changes in the levels of expression of outer membrane proteins due to phase variation and, in the case of TprK, antigenic variation as a result of intra-genomic gene conversion (9) (36) (59).

6 DIAGNOSIS

It is based on clinical characteristics and diagnostic techniques, such as direct microscopic examination, used when lesions are present; non-treponemal tests, used for screening; confirmatory treponemal tests; and direct detection of the microorganism, currently used in research laboratories (32). Before giving birth, ultrasound has proven to be a useful tool for diagnosing congenital syphilis (25). The diagnosis of the newborn requires the correlation of the findings of the pregnancy product with the background of syphilis in the mother, the gestational age of contagion of the infection and of reception of adequate treatment (60) (61).

6.1 DIRECT TESTS

The rabbit infectivity test or RIT is the only standardized method for the isolation of T. pallidum. This technique has the highest sensitivity to detect treponemes; therefore, it is considered the gold standard at the time of evaluating the sensitivity of a diagnostic test. It is based on the inoculation of the sample in the testis, scrotum, venous or eye Rabbit lines and tissue maceration of a localized syphilitic lesion to corroborate the presence of the microorganism. This test is limited because it requires the use of live animals and the direct manipulation of Treponema pallidum, therefore it is not used in routine clinical laboratories (34) (62).

Dark field microscopy is a less sensitive method. It requires special equipment (dark field microscopy), trained and experienced laboratory technicians, and it has a specificity of 97% and a
sensitivity of 78-86%. This technique confirms the presence of spirochetes in skin and mucosal lesions such as those found in the oral cavity in children under 6 months of age (63) (64).

6.2 SEROLOGICAL TESTS

Non-treponemal tests: They are based on antigens composed of alcoholic solutions with predetermined amounts of cardiolipins, cholesterol and lecithins. They measure substances simultaneously, which are produced in tissues damaged by *T. pallidum* (e.g. reagins) or by other diseases. They do not measure specific antibodies against *T. pallidum*, therefore their positivity does not ensure syphilitic disease (21). All non-treponemal tests can present prozone phenomena (false negatives), when the samples are strongly reactive, so it is convenient to carry out titrations. One of the most commonly used non-treponemal tests for the presumptive diagnosis of syphilis is the Venereal Disease Research Laboratory (V.D.R.L), which is a flocculation reaction. On the other hand, the agglutination test R.P.R can be used with serum and plasma using antigen bound to carbon particles (19) (33).

Treponemal tests: they are used to confirm positive screening tests. They detect specific antibodies against *Treponema pallidum*; they are expensive tests, require equipment and technical expertise and, therefore, are not widely available. False-positive rates are slightly lower for treponemal tests in contrast to non-treponemal tests (63) (65). This type of tests includes the FTA-ABS 200 DS (indirect immunofluorescence with absorption and double staining), which uses as antiserum an IgG labeled with tetramethylrhodamine isothiocyanate and an antitreponema serum labeled with fluorescein isothiocyanate as contrast. The TPHA (Microhemaglutination) test uses erythrocytes sensitized with Treponema Nichols strain antigens and Reiter strain absorber. The Captia Syphilis M (ELISA capture anti heavy chain) test is performed in serum and its greatest use is focused on the diagnosis of congenital syphilis, especially the symptomatic type, since it seems to be the methodology with greater sensitivity for the detection of this class of immunoglobulin (66) (67).

Serology as a diagnostic method in congenital syphilis is used in parallel with the serum of the mother and the baby at the time of the birth; a title of four times or more in the newborn's serum over the mother's title is very suggestive of congenital infection. However, if this result does not occur, congenital infection is not excluded given the fact that the mother may have been infected in a period close to the birth and the antibody concentration is still not high enough, since a serological response to the presence of *T. pallidum* usually takes between 1-4 weeks to develop (67) (68).

The main problem with the serological diagnosis of congenital syphilis is to distinguish the infected asymptomatic infant from the uninfected infant, born from a mother with reactive serological
tests for syphilis. This difficulty is due in large part to the inability to distinguish the humoral immune response of the mother from the response of the newborn's specific antibodies, since the antibodies of immunoglobulin G type cross through the placenta to the fetus (67) (69).

In recent years, a quick treponemal test has been implemented, which can scale in the selection process in facilities where traditional tests are not available. According to the studies performed by Tucker et al., these syphilis tests showed high sensibility (75%-94%) and good specificity (98%-99%); they are both comparable to the characteristics of the detection tests used nowadays for different infections from the ones of treponema. Nonetheless, additional research is required in cases of patients infected with HIV for the effective deployment of syphilis screening programs. These tests are simple, economical and do not require refrigeration or highly trained laboratory staff to perform them (41) (70).

6.3 MOLECULAR TESTS

Among the genes that have been used for the molecular determination of gestational and congenital syphilis are the following: 16S rRNA, polA, tpr, arp, TpN47, tpf-1, bmp, tmpA and tmpB. On the other hand, outer membrane proteins such as Tp33, Tp37, Tp39, Tp43, Tp97.3 and especially Tp15, Tp17, Tp45 and Tp47 are highly antigenic, since they are directly involved with the pathogenesis of the microorganism and therefore have been considered as potential markers for the serological detection of the disease through its use as recombinant proteins with an excellent level of sensitivity and diagnostic specificity (41) (42) (71).

The molecular detection of the spirochete in body fluids and tissues has an enormous attraction as a diagnostic strategy for syphilis, since it provides evidence of active infection. Additionally, it is an early diagnosis for those patients who are in the window period and for those who cannot attend to sequential serological analysis (71) (72). On the other hand, PCR tests can easily distinguish between T. pallidum and B. burgdorferi; this is important because cross-reactivity between these two spirochetes is a major problem in the serological diagnostic tests of syphilis and Lyme disease (72). Several variants of PCR have been used in the diagnosis of gestational and congenital syphilis. In previous studies, Pinilla et al. evaluated the chain reaction of the conventional polymerase, nested and in real time, aimed at different molecular targets. For the polA and 16S rDNA genes, they achieved sensitivity of 70% by means of endpoint PCR; and regarding the TpN47 gene, sensitivity reached by PCRn (nested) and PCR-RT (real time) was 90% and 100% respectively; also specificities ranging between 70% and 100% were reached (43) (73). On the other hand, Palmer et al. reported a limit of detection of 1 pg of purified DNA of T. pallidum, which represents approximately 800 copies
of the genome, using as a target the gene codifying for the integral membrane lipoprotein of 47 KDa (71).

PCR can show a negative result with reactive prior serology as it is evidenced in the Graig Tipple study; perhaps due to the presence of maternal antibodies in the neonatal circulation. Moreover, it is important to take into account that serological techniques such as VDRL and RPR, detect reaginic antibodies and non-treponemal antibodies, which means that these serologies can be reactive with other pathologies such as measles, varicella, hepatitis, infectious mononucleosis, leprosy, tuberculosis, malaria and autoimmune diseases (31) (74).

TpN47 gene has shown good results in terms of sensitivity and specificity (43) (73). On the other hand, 16S rDNA and polA genes could be used for molecular typing that emerges as an important tool to monitor the appearance of macrolide-resistant strains, evaluate the subtypes associated with the central nervous system condition, differentiate infection and re-infection processes, as well as understanding the transmission of T. pallidum and the epidemiological development of the disease. For this same purpose, the arp (acidic repeat protein) gene and the family of tpr subfamily II genes (tprE, tprG and tprJ) have been used A joint study with the rpsA and tp0548 genes has also been adopted to increase the discriminatory capacity of the typing of the strains (74) (75).

7 TREATMENT

Penicillin G Benzathine treatment is intramuscular in a single dose for primary, secondary and early latent syphilis. It is applied for 3 weeks in the case of late or undetermined latent syphilis and tertiary syphilis (32). Aqueous Crystalline Penicillin G is the medicine of choice for the treatment of neurosyphilis. Pregnant women who have a history of allergy should be desensitized and treated with penicillin (4), if this does not prove effective, Erythromycin may be used, but at birth, the baby should receive immediate treatment since this antibiotic does not cross the placenta (76). Infants should be treated with IV aqueous crystalline penicillin G for a total of 10 to 14 days at 50,000 U/kg per dose every 12 hours (100,000 U/kg/day) during the first 7 days of life and every 8 hours during days of life 8 to 30 (150,000 U/kg/day) (34).

There have not been any reports so far regarding the presence of mobile elements in T. pallidum; however, several PBP (penicillin binding proteins) have been found. The most abundant lipoprotein in the Tp47 membrane presents a covalent modification, acts as a PBP and has b-lactamase activity that paradoxically is inhibited by the products of the reaction. Although T. pallidum remains sensitive to penicillin, the appearance of natural resistance in T. denticola, the scarce difference of
Tp47 with other treponemal b-lactamases and the phylogenetic similarity suggest a possible mutation, diffusion and acquisition of resistance to penicillin in the future (74) (77).

8 PERSPECTIVES

Finally, the definitive elimination of syphilis will require the development of an effective vaccine and for such purpose Smith B et al. conducted in-vivo research with previously irradiated treponemes, maintained at 4°C, in combination with recombinant or native proteins such as 4kD, 6pd, TmpB, TprK, showing relative success in the design of a possible vaccine against Treponema. However, recombinant proteins such as Tp0326, Tp0453, and a chimera Tp0453-Tp0326, have not been studied in population (78).

The pharmaceutical industry is currently developing new technologies with the purpose of contributing to the eradication of syphilis; as it is the case of a reverse vaccine, based on the prediction of the antigenic properties of *T. pallidum* proteins through bioinformatic analysis in order to select potential candidates for in silico vaccines. In 2017, Lithgow et al. evaluated the potential of the vaccine Tp0751, a vascular adhesion of *Treponema pallidum* where animals immunized with Tp0751 exhibited a significant reduction of the bacterial load in organs after exposure to *T. pallidum*, in contrast to the animals that had not been immunized. These findings provide evidence that Tp0751 could be a promising candidate for the syphilis vaccine (38) (39).

Therefore, molecular techniques can be considered as validated tools in biological samples with optimum efficiency, sensitivity and specificity that will allow the timely detection of this disease and the adoption of pertinent measures, contributing to the mitigation of the economic, social and health consequences of syphilis in the world (79, 80).
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