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Placental Syphilis: A Comprehensive Review of Routine Histomorphology, HIV Co-infection, Penicillin Treatment, Immunohistochemistry, and Polymerase Chain Reaction

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ABSTRACT

Introduction: Placental examination is valuable for diagnosing congenital syphilis, but the classic histological triad is not always observed. This study aimed to identify additional morphological clues, evaluate the sensitivity of IHC and qPCR, and investigate the impact of HIV co-infection and penicillin treatment on placental morphology. Materials and methods: Two hundred and fifteen placental specimens with treponemal infection were reviewed. Morphological findings, IHC, and qPCR results were analyzed. Results: Chronic villitis (94%), acute chorioamnionitis (91.6%), and villous immaturity (65.6%) were the most common abnormalities. HIV co-infection and penicillin treatment were associated with reduced frequencies of inflammatory lesions. IHC and qPCR exhibited sensitivities of 74.4 and 25.8%, respectively, confirming the diagnosis in 42 cases with negative or unknown serology. Conclusion: Villitis, chorioamnionitis, and villous immaturity were identified as the predominant placental abnormalities. HIV co-infection and penicillin treatment can impact morphology and hamper the diagnosis. IHC and q-PCR are valuable adjuncts when serology is negative.

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Introduction

Syphilis, caused by the bacterium *Treponema pallidum* ssp. *pallidum* is the second leading cause of stillbirth worldwide [1]. The global incidence increased by 1.7% annually between 2010 and 2019, whilst the South African prevalence among antenatal clinic attendees increased from 1.5% to 2.6% [2,3]. South Africa has a high burden of human immunodeficiency virus (HIV) with a national prevalence of 30% among antenatal clinic

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attendees in 2019 [4]. In 2015, a South African antenatal survey showed that 51.5% of HIV-positive mothers were co-infected with syphilis [5]. During the primary stage of disease, the risk of fetal transmission is 70–100% and increases with advancing gestational age [6–9]. Infection may lead to spontaneous abortion, non-immune hydrops, preterm birth, low birth weight, early and late congenital syphilis, and neonatal death. Seventy percent of untreated women will have an adverse pregnancy outcome [6–8,10,11]. HIV-associated adverse pregnancy outcomes include prematurity and low birth weight [12]. Maternal diagnosis is made using non-treponemal and treponemal serological tests like rapid plasma reagin (RPR), with high sensitivity (95–100%) and specificity (81–96%) [13,14]. False negative results can occur in HIV infection, pregnancy, and the prozone phenomenon [15–17]. Antenatal ultrasound and invasive sampling techniques aid in fetal diagnosis [6,7,9,18,19]. Maternal treatment with intramuscular benzathine benzylpenicillin (BPG) injections has over 98% efficacy [6,7,9,11].

Placental evaluation

Approximately 60% of infants are asymptomatic at delivery and if left untreated, may only develop clinical manifestations months or years later. Serological confirmation can be challenging as maternal Immunoglobulin G (IgG) antibodies transferred to the fetus may be detected for up to 18 months [11]. Early diagnosis is crucial to prevent the morbidity and mortality associated with congenital syphilis [6,7,11,20]. Placental examination is a valuable diagnostic tool, especially when clinical and serological diagnostic criteria are inconclusive. A definitive diagnosis is made with the demonstration of the organism in fetal, neonatal, or placental tissue [21–24]. Placental evaluation may increase the diagnostic frequency up to 89% in live-born and 97% in stillborn infants, compared to 67 and 92% in cases relying on clinical and serological findings alone [25]. Infected placentas are reported to often exhibit placentomegaly and a suggestive triad of histological features: villous enlargement and hypercellularity; proliferative vasculopathy and acute and/or chronic villitis [18,21,23–28]. In HIV-positive individuals, placental findings often include low placental weight, maternal vascular malperfusion, and acute chorioamnionitis, with less frequent chronic villitis [12,28]. Immunohistochemistry (IHC) can demonstrate *Treponema pallidum* spirochetes in formalin-fixed and paraffin-embedded (FFPE) tissue. Studies on mucocutaneous lesions showed rates of positivity of 67, 55, and 13% in primary, secondary, and tertiary syphilis, respectively [29,30]. HIV status has not been reported to have a significant effect on the immunohistochemical detection of infectious organisms [31]. Polymerase chain reaction (PCR) remains an effective detection method, despite deoxyribonucleic acid (DNA) degradation in FFPE tissue, with sensitivity of 26–75 and 100% specificity, depending on the target gene and stage of disease [13,29,30,32]. Placental specimens had positivity rates of 18–87%, depending on the target gene, detection method, and tissue type (umbilical cord or parenchymal sections) [23,24].

Rationale

Given the increasing prevalence and significant morbidity and mortality related to congenital syphilis, early diagnosis is essential. Our study examines the largest cohort

of placentas with syphilis to date and aims to describe routine pathological features and potentially identify additional clues that can assist in making the diagnosis. Our setting is ideal for evaluating the influence of HIV and antiretroviral treatment (ART) on placental morphology. We also determine the morphological effects of BPG treatment and determine the sensitivity of *T. pallidum* IHC and qPCR in placental tissue.

Materials and methods

Case selection

A retrospective laboratory-based study was conducted in the Division of Anatomical Pathology, Stellenbosch University, and National Health Laboratory Service (NHLS) at Tygerberg Hospital, Cape Town, South Africa. The study focused on placental specimens diagnosed with syphilis between 2010 and 2020. Relevant cases were identified by searching the electronic laboratory information system using SNOMED clinical codes. The macroscopic examination and sampling of the specimens was performed using established criteria from the Amsterdam Placental Workshop Group Consensus Statement [33]. Ethical approval was obtained from the Health Research Ethics Committee (reference number S20/09/238), with a waiver of informed consent. Clinical information was collected from laboratory request forms and clinical records. Serology results were obtained from the laboratory result platforms. Archived hematoxylin and eosin-stained (H&E) sections, *T. pallidum* IHC sections, and FFPE tissue blocks were retrieved for analysis.

Evaluation of placental pathology

Two authors (Y.A.M, P.T.S.) reviewed the sections and recorded morphological findings on a data sheet. The evaluation followed established criteria from the Amsterdam Placental Workshop Group Consensus Statement [33]. Placental weight was obtained from the pathology report after reviewing the slides. Placental weight and villous maturation were compared to the expected values for gestational age using reference values published by Pinar et al. [34]. Villous maturation was assessed by evaluating villous morphology, proportions of different villous types, villous vasculature, and syncytial knots.

Treponema pallidum immunohistochemistry

Immunohistochemical staining was performed on 3 µm thick, FFPE sections showing the most significant pathology in each case using *T. pallidum* antibody (397A series rabbit polyclonal antibody, Cell Marque, Rocklin, CA, USA) on an automated staining platform (Bond III, Leica Biosystems, Buffalo Grove, IL, USA) according to the previous internal laboratory optimized protocol using 3,3′-diaminobenzidine (DAB) chromogen. The immunostained slides were interpreted by two authors (Y.A.M., P.T.S.).

Treponema pallidum real-time polymerase chain reaction

Samples and DNA extraction

Eighty cases were selected for real-time PCR (qPCR) based on available funds. The selected cases included all cases with negative IHC results and randomly selected cases with positive IHC results from each year. The same FFPE tissue block used for IHC was sent to the Molecular Pathology Department at Lancet Laboratories in Richmond, Johannesburg, South Africa. DNA extraction took place on the MagNA Pure® 96 instrument utilizing the FFPE Plus DNA protocol. The turnaround time for qPCR results was 48–72 h.

Molecular identification of *Treponema pallidum* spp. *pallidum*

Real-time PCR was conducted using the PrimerDesign genesig kit, targeting the polA gene for molecular identification of *T. pallidum*. The primers demonstrated 100% homology with over 95% of the NCBI database reference sequences and >95% priming efficiency. The kit's sensitivity allowed detection of <100 copies of the target template under optimal PCR conditions. Negative controls consisted of RNase/DNase free water as the DNA template, whilst positive controls utilized the provided positive control template. Fluorescence signals were measured in the FAM (465–510) channel using a Roche® LightCycler 480 analyzer. Concurrently, the ACTB gene was detected using a separate PrimerDesign genesig kit as an internal control for tissue samples, following the same protocol.

Statistical analysis

Data analysis was conducted using Stata/MP 17 and SPSS v28 statistical analysis software. Frequency tables were used to present the data, including numbers, percentages, means, and medians to assess results distribution. Associations between categorical variables were determined using Pearson's Chi-squared test and Fisher's exact test. A p-value of <0.05 was considered significant. The sensitivity of *T. pallidum* IHC and PCR tests was assessed to evaluate their accuracy, with serology serving as the gold standard.

Study limitations

Incomplete clinical and serological data were observed in some cases. For analyses requiring complete data, only cases with available information were included.

Results

A total of 225 placentas from 222 specimens were initially identified. Three specimens consisted of dichorionic diamniotic twin placentas, and each twin's morphology was evaluated separately. Three cases were excluded due to insufficient tissue, severe autolysis, and severe intrauterine fetal death (IUFD) associated changes with tombstone villi, which prevented morphological assessment. Seven cases were excluded as the diagnosis could not be confirmed through serology, IHC, or qPCR. Of the remaining

215 placentas, 4 (1.9%) cases lacked umbilical cord sections, whilst 2 (0.9%) cases lacked placental membrane sections. Five (2.3%) cases had only two parenchymal sections. A total of 78.1% of cases resulted in IUFD and in 10.7% of cases the outcome was unknown. Table 1 summarizes the most important demographic and pregnancy-related information, serology results, and treatment-related information.

Placental pathology

Table 2 and Figure 1 summarize the key morphological findings observed during routine examination. Placentomegaly (placental weight >90th centile) was present in 56.4% of cases, whilst no other significant macroscopic features were identified. A total of 61 (28.4%) cases exhibited the classical histological triad of immature hyper cellular villi, proliferative vasculopathy, and acute and/or chronic villitis. Thirteen (6.1%) cases displayed a previously described triad of features including immature hypercellular villi, proliferative vasculopathy, and necrotizing funisitis[35]. [Immature villi, proliferative vasculopathy, and chronic villitis were observed in 65.6, 52.1, and 94% of cases, respectively. Histiocytic villitis was the most common primary pattern of villitis, accounting for 96.5% of cases. Secondary and tertiary patterns of villitis were observed in 45.6 and 20.9% of cases, respectively, with acute villitis comprising 66.3 and 35.6% of cases in each category. Chronic villitis was more commonly observed in cases with villous immaturity ($p = .015$). Proliferative vasculopathy was more fre quently associated with acute chorioamnionitis, chronic villitis, stem villous villitis, and villous immaturity ($p = .008$, <.001, .004, and .003, respectively). Other

Table 1. Demographic, pregnancy, serological, and treatment information.

Category	(n)	Mean (std)[a]	Median [IQR][b]	Min/max
Maternal age in years	(212)	24.7 (4.9)	24 [21; 27]	15/41
Gravidity	(182)	2.2 (1.3)	2 [1; 3]	1/8
Parity	(182)	1.7 (1.1)	1 [1; 2]	0/7
Gestation in weeks	(156)	31.2 (4.5)	31.5 [29; 34]	14/41
Category	**(n)**	**Variable**	**Total (%)**	
Pregnancy outcome	(215)	IUFD[c]	168 (78.1)	
		Neonatal death	11 (5.1)	
		Survivor >28 days	13 (6.1)	
		Unknown	23 (10.7)	
Syphilis serology result	(212)	Negative	22 (10.4)	
		Positive	166 (78.3)	
		Unknown	24 (11.3)	
BPC[d] treatment before delivery	(212)	Not received	103 (48.6)	
		Received	21 (9.9)	
		Unknown	88 (41.5)	
HIV[e] status	(212)	Negative	126 (59.4)	
		Positive	36 (17.0)	
		Unknown	50 (23.6)	
ART[f] status	(36)	Not on treatment	5 (13.9)	
		On treatment	17 (47.2)	
		Unknown	14 (38.9)	

[a]Standard deviation.
[b]Interquartile range.
[c]Intrauterine fetal death.
[d]Benzathine benzylpenicillin.
[e]Human immunode ciency virus.
[f]Antiretroviral treatment.

Table 2. Morphology on routine examination.

Category	(n)	Mean (std[a])	Median [IQR[b]]	Min/max
Placental weight in grams	(212)	450.2 (166.7)	432.5 [326; 550]	98/1040
Category	**(n)**	**Variable**	**Value n (%)**	
Placental weight for GA[c]	(156)	<10th centile	19 (12.2)	
		Below mean	25 (16.0)	
		Above mean	24 (15.4)	
		>90th centile*	88 (56.4)	
Funisitis	(211)	Absent	140 (66.4)	
		Present	71 (33.6)	
Necrotizing funisitis	(211)	Absent	156 (73.9)	
		Present	55 (26.1)	
Villous maturation for GA	(157)	Concordant	33 (21.0)	
		Immature*	103 (65.6)	
		Accelerated	21 (13.4)	
Erythroblastosis	(215)	Absent	123 (57.2)	
		Present	92 (42.8)	
Acute chorioamnionitis	(215)	Absent	18 (8.4)	
		Present†	197 (91.6)	
Maternal response	(197)	Absent	24 (12.2)	
		Present	173 (87.8)	
		Stage 1: 43 (24.8)	Grade 1: 68 (39.3)	
		Stage 2: 79 (45.7)	Grade 2: 105 (60.7)	
		Stage 3: 51 (29.5)		
Fetal response	(197)	Absent	56 (28.4)	
		Present	141 (71.6)	
		Stage 1: 53 (37.6)	Grade 1: 43 (30.5)	
		Stage 2: 26 (18.4)	Grade 2: 98 (69.5)	
		Stage 3: 62 (44.0)		
Villitis†	(215)	Absent	13 (6.0)	
		Present†	202 (94.0)	
Primary pattern	(202)	Histiocytic*	195 (96.5)	
		Lymphocytic	6 (3.0)	
		Lymphoplasmacytic	1 (0.5)	
Extent	(202)	Focal	25 (12.4)	
		Diffuse	177 (87.6)	
Type if histiocytic	(195)	Pattern A	159 (81.5)	
		Pattern B	36 (18.5)	
Secondary pattern of villitis	(215)	Absent	117 (54.4)	
		Present	98 (45.6)	
Tertiary pattern of villitis	(215)	Absent	170 (79.1)	
		Present	45 (20.9)	
Intervillous abscess	(215)	Absent	195 (90.7)	
		Present	20 (9.3)	
Proliferative vasculopathy	(215)	Absent	103 (47.9)	
		Present†	112 (52.1)	
Deciduitis	(215)	Absent	189 (87.9)	
		Present	26 (12.1)	

[a]Standard deviation.
[b]Interquartile range.
[c]Gestational age.
*Most common findings on routine evaluation.
†Includes acute and chronic villitis.

inflammatory lesions, such as funisitis, acute chorioamnionitis, and deciduitis, were observed in 33.6, 91.6, and 12.1% of cases, respectively. Acute chorioamnionitis was associated with a higher frequency of IUFD ($p = .025$). Lymphocytic and plasmacytic deciduitis showed similar frequencies, with a slightly higher frequency of decidual lymphocytes. Necrotizing funisitis was present in 77.5% of cases with funisitis and 26.1% of cases overall. Umbilical cord thrombosis and fibrin thrombi in the chorionic

Figure 1. Morphological findings on routine examination. (A) Necrotizing funisitis involving both umbilical arteries. (B) Necrotizing vasculitis of a chorionic vessel. (C) Acute villitis. (D) Focus on lymphocytic (top) and granulomatous (bottom) villitis. (E) Lymphoplasmacytic villitis. (F) Histiocytic villitis and acute vasculitis of a stem villous. (G) Large intervillous abscess. (H) Proliferative vasculopathy showing fibromuscular sclerosis of a villous vessel. (I) Fibrin thrombus formation in a vessel of the chorionic plate [Hematoxylin & Eosin, original magnifications ×2 (A,B), ×20 (I), ×40 (G), ×100 (F), ×200 (C,D,H), ×400 (E)].

plate were more commonly associated with necrotizing funisitis ($p = .017$ and .008, respectively). All instances of umbilical cord thrombosis occurred in pregnancies with IUFD, although this was not found to be statistically significant.

Histiocytic villitis

Two distinct patterns of histiocytic villitis were observed. Pattern A (classic pattern) displayed prominently enlarged villi with abundant Hofbauer cells that were easily identified on low magnification (Figs. 2(A–C)). In contrast, pattern B exhibited subtle

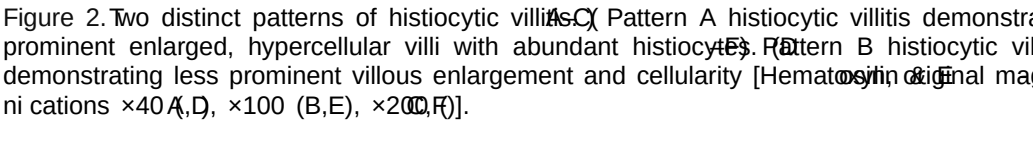


Figure 2. Two distinct patterns of histiocytic villitis (A–C). Pattern A histiocytic villitis demonstrating prominent enlarged, hypercellular villi with abundant histiocytes (A–C). Pattern B histiocytic villitis demonstrating less prominent villous enlargement and cellularity [Hematoxylin & Eosin, original magnifications ×40 (A,D), ×100 (B,E), ×200 (C,F)].

villous enlargement and a mild to moderate increase in Hofbauer cells (Figs. 2(D–F)). Pattern B was associated with lower frequencies of villous immaturity and proliferative vasculopathy compared to pattern A ($p = .026$ and $<.001$, respectively), and was less commonly diffuse in extent ($p = <.001$). Secondary and tertiary patterns of villitis ($p < 0.001$ and 0.001, respectively) and stem villous villitis ($p = <.001$) were also less frequent. Pattern A was more commonly associated with acute villitis as a secondary pattern (70%), while pattern B was associated with lymphocytic villitis (66.7%). Intervillous abscesses were absent in cases with pattern B ($p = .028$). Although pattern B showed lower frequencies of chorioamnionitis ($p = .017$), the fetal inflammatory response was more severe with higher stage and grade ($p = .018$ and .006, respectively) compared to pattern A. Pattern B was also associated with higher frequencies of funisitis,

Table 3. Comparative analysis based on the pattern of histiocytic villitis.

			Histiocytic villitis		
Category	(n)	Variable	Pattern A	Pattern B	p-value (test)
Funisitis	(191)	Absent	114 (73.1)	16 (45.7)	.002 (PCS[a])
		Present	42 (26.9)	19 (54.3)	
Necrotizing funisitis	(191)	Absent	122 (78.2)	21 (60.0)	.025 (PCS)
		Present	34 (21.8)	14 (40.0)	
Villous maturation for GA[b]	(142)	Concordant	20 (17.4)	8 (29.6)	.026 (PCS)
		Immature	85 (73.9)	13 (48.2)	
		Accelerated	10 (8.7)	6 (22.2)	
Acute chorioamnionitis	(195)	Absent	7 (4.4)	6 (16.7)	.017 (FE[c])
		Present	152 (95.6)	30 (83.3)	
Fetal response stage	(128)	Stage 1	49 (47.1)	4 (16.7)	.018 (PCS)
		Stage 2	18 (17.3)	5 (20.8)	
		Stage 3	37 (35.6)	15 (62.5)	
Fetal response grade	(128)	Grade 1	39 (37.5)	2 (8.3)	.006 (PCS)
		Grade 2	65 (62.5)	22 (91.7)	
Primary villitis extent	(195)	Focal	7 (4.4)	12 (33.3)	<.001 (FE)
		Diffuse	152 (95.6)	24 (66.7)	
Secondary pattern of villitis	(195)	Absent	69 (43.4)	30 (83.3)	<.001 (PCS)
		Present	90 (56.6)	6 (16.7)	
Tertiary pattern of villitis	(195)	Absent	116 (73.0)	35 (97.2)	.001 (PCS)
		Present	43 (27.0)	1 (2.8)	
Intervillous abscess	(195)	Absent	139 (87.4)	36 (100)	.028 (FE)
		Present	20 (12.6)	0 (0.0)	
Stem villous villitis	(195)	Absent	3 (1.9)	9 (25.0)	<.001 (FE)
		Present	156 (98.1)	27 (75.0)	
Proliferative vasculopathy	(195)	Absent	56 (35.2)	28 (77.8)	<.001 (PCS)
		Present	103 (64.8)	8 (22.2)	

[a]Pearson's chi-squared.
[b]Gestational age.
[c]Fisher's exact.

particularly necrotizing funisitis ($p = .002$ and .025, respectively). Table 3 summarizes the significant findings associated with pattern B histiocytic villitis.

Twin placentas

Differences in morphology were observed among the respective placentas of two out of the three dichorionic diamniotic twin pairs (66.7%). These differences included variations in the presence of maternal and fetal response in chorioamnionitis, proliferative vasculopathy, and secondary patterns of villitis. None of these findings were statistically significant.

HIV status

Out of the 162 placentas with known HIV status, 36 (22.2%) were from HIV-positive mothers, who were more likely to have received BPG treatment ($p = .019$). HIV infection was associated with higher frequencies of pattern B histiocytic villitis and fibrin thrombi in the chorionic plate ($p = .053$ and .042, respectively). In cases of necrotizing funisitis, HIV positive cases less commonly showed involvement of all three umbilical vessels ($p = .024$). HIV-positive cases also exhibited no distal villous edema ($p = .025$). Table 4 provides a summary of the noteworthy findings observed among HIV-negative and HIV-positive cases.

Table 4. Comparative analysis based on HIV status.

Category	(n)	Variable	HIV[a] status: Negative	HIV[a] status: Positive	p-value (test)
BPG[b] treatment status	(118)	Not treated	86 (88.7)	14 (66.7)	.019 (FE[c])
		Treated	11 (11.3)	7 (33.3)	
Vessels involved by[d] NF	(42)	Vein	6 (18.7)	4 (40.0)	.024 (FE)
		One artery	7 (21.9)	2 (20.0)	
		Two arteries	1 (3.1)	3 (30.0)	
		Vein and artery	8 (25.0)	0 (0.0)	
		All vessels	10 (31.3)	1 (10.0)	
Type of histiocytic villitis	(147)	Pattern A	99 (86.8)	24 (72.7)	.053 (PC[e]S)
		Pattern B	15 (13.2)	9 (27.3)	
Fibrin thrombi in CP[f]	(164)	Absent	125 (97.7)	32 (88.9)	.042 (FE)
		Present	3 (2.3)	4 (11.1)	
Distal villous edema	(164)	Absent	111 (86.7)	36 (100)	.025 (FE)
		Present	17 (13.3)	0 (0.0)	

[a]Human immunodeficiency virus.
[b]Benzathine benzylpenicillin.
[c]Fisher's exact.
[d]Necrotizing funisitis.
[e]Pearson's chi-squared.
[f]Chorionic plate.

Table 5. Comparative analysis based on antiretroviral treatment status.

Category	(n)	Variable	ART[a] treatment: Treated	ART[a] treatment: Not treated	p-value (test)
Secondary pattern of villitis	(22)	Absent	7 (41.2)	5 (100)	.040 (FE[b])
		Present	10 (58.8)	0 (0.0)	
Proliferative vasculopathy	(22)	Absent	6 (35.3)	5 (100)	.035 (FE)
		Present	11 (64.7)	0 (0.0)	
Deciduitis	(22)	Absent	15 (88.2)	2 (40.0)	.055 (FE)
		Present	2 (11.8)	3 (60.0)	

[a]Antiretroviral treatment.
[b]Fisher's exact.

HIV treatment

Antiretroviral treatment information was available in 22 of the HIV-positive cases. Untreated cases had no secondary patterns of villitis or proliferative vasculopathy ($p = .040$ and .035, respectively), but a higher frequency of deciduitis ($p = .055$). The morphological findings in cases receiving ART resembled those in cases from HIV-negative mothers. The duration of ART and viral load had no statistically significant effect on morphology. A CD4 count above 200 cells/mm^3 was associated with a lower frequency of deciduitis ($p = .034$). Table 5 provides a summary of the noteworthy findings in HIV-treated and untreated cases.

Benzathine benzylpenicillin treatment

Treatment information was available for 124 cases, including 21 cases that received at least one dose of BPG before delivery. Fifteen of the 21 cases (71.4%) had a positive treponemal serology result at delivery or postpartum. In a single case, postpartum serology was negative, whereas in the remaining cases, the serology status was unknown. Treated cases exhibited lower frequencies of villitis ($p < .001$), acute chorioamnionitis,

Table 6. Comparative analysis based on benzathine benzylpenicillin treatment status.

			BPG[a] treatment		
Category	(n)	Variable	Not treated	Treated	p-value (test)
Non-necrotizing funisitis	(125)	Absent	100 (96.1)	17 (80.9)	.027 (FE[b])
		Present	4 (3.9)	4 (19.1)	
Vessels involved by[c] NF	(31)	Vein	6 (21.4)	0 (0.0)	.035 (FE)
		One artery	8 (28.6)	0 (0.0)	
		Two arteries	1 (3.6)	1 (33.3)	
		Vein and artery	5 (17.8)	2 (66.7)	
		All vessels	8 (28.6)	0 (0.0)	
Villitis	(126)	Absent	3 (2.9)	7 (33.3)	<.001 (FE)
		Present	102 (97.1)	14 (66.7)	

[a]Benzathine benzylpenicillin.
[b]Fisher's exact.
[c]Necrotizing funisitis.

and necrotizing funisitis involving all umbilical vessels ($p = .035$). Treatment was associated with higher frequencies of non-necrotizing funisitis ($p = .027$). The noteworthy morphological findings in untreated and treated cases are summarized in Table 6.

Immunohistochemistry

Immunohistochemistry conducted on the 215 selected sections, showed positive IHC results in 76.7% of cases, resulting in a sensitivity of 74.4% (95% CI: 67.1–80.8%) compared to serology. Parenchymal sections were positive in 74.9% of cases, whilst tests on the umbilical cord yielded positive results in 89.3% of cases (Figs. 3(A,B)). Spirochetes were mainly centered closer to the umbilical vessel lining (Fig. 3(B)). IHC confirmed the presence of the organism in 41 cases with negative or unknown serology. Higher frequencies of IHC positivity were observed in cases with villitis ($p = .014$), a secondary and tertiary pattern of villitis ($p < .001$ and .003, respectively), stem villous villitis ($p = .005$), and proliferative vasculopathy ($p = .001$). Lower frequencies of positivity were observed in cases submitted before 2014 ($p = .034$), HIV untreated cases ($p = .021$), cases treated with BPG ($p = .007$), cases with non-necrotizing funisitis ($p = .003$), cases with a higher grade of maternal response in acute chorioamnionitis ($p = .048$), cases with severe IUFD changes ($p = .006$), and cases with pattern B-histiocytic villitis ($p < .001$).

Two artifacts were observed that could potentially lead to incorrect interpretation of the IHC stain (Figs. 3(C,D)): formalin pigment as small brown-black granular deposits around blood vessels and erythrocytes, and rod-like refractile golden-brown structures resembling spirochetes on low magnification but lacking the spiraled architecture of true spirochetes. Close scrutiny was necessary to avoid false-positive results in these cases. Significant associations are summarized in Table 7.

Real-time polymerase chain reaction

Seven out of the 80 cases submitted for PCR were excluded as the diagnosis could not be confirmed with serology, IHC, or PCR. Among the remaining 73 selected cases,

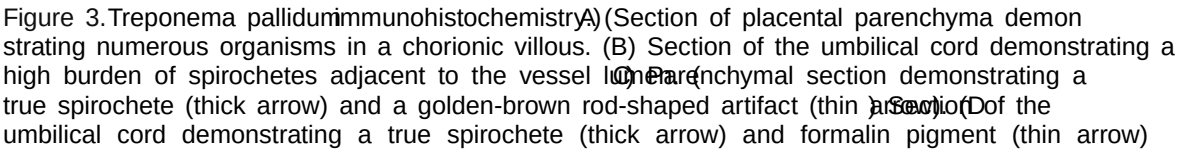


Figure 3. Treponema pallidum immunohistochemistry. (A) Section of placental parenchyma demonstrating numerous organisms in a chorionic villous. (B) Section of the umbilical cord demonstrating a high burden of spirochetes adjacent to the vessel lumen. (C) Parenchymal section demonstrating a true spirochete (thick arrow) and a golden-brown rod-shaped artifact (thin arrow). (D) Section of the umbilical cord demonstrating a true spirochete (thick arrow) and formalin pigment (thin arrow) [Original magnification ×400 (A–D)].

17 (23.3%) cases tested positive for Treponema pallidum DNA, resulting in a sensitivity of 25.8% (95% CI: 15.5–38.5%) when compared to serology. Of the 43 selected cases that tested negative for IHC, 7 (16.3%) cases were positive on PCR, and of the 23 randomly selected cases that tested positive for IHC, 10 (43.5%) cases were positive on PCR. Most PCR tests were performed on parenchymal sections, which showed positive results in 16.9% of cases, whilst tests on the umbilical cord yielded positive results in 75% of cases. Cases associated with necrotizing funisitis ($p = .014$), maternal response in acute chorioamnionitis ($p = .009$), and positive IHC ($p = .006$) showed higher frequencies of positivity on qPCR. One additional case that had negative

Table 7. Treponema pallidum immunohistochemistry analysis.

Category	Variable	Result	95% CI[a]	
IHC[b] diagnostic test analysis	Sensitivity	74.4%	67.1%	80.8%
	Specificity	8.7%	1.1%	28.0%
	Positive predictive value	85.6%	78.9%	90.9%
	Negative predictive value	4.4%	0.5%	15.2%

Category	(n)	Variable	Total (%)
IHC result	(215)	Negative	50 (23.3)
		Positive	165 (76.7)

			IHC result		
Category	(n)	Variable	Negative	Positive	p-value (test)
Test performed on	(215)	Cord	3 (6.0)	25 (15.2)	.092 (PCS[c])
		Parenchyma	47 (94.0)	140 (84.8)	
ART[d] status	(22)	On treatment	3 (42.9)	14 (93.3)	.021 (FE[e])
		Not on treatment	4 (57.1)	1 (6.7)	
BPG[f] treatment status	(126)	Not treated	21 (67.7)	84 (88.4)	.007 (PCS)
		Treated	10 (32.3)	11 (11.6)	
Non-necrotizing funisitis	(211)	Absent	39 (81.2)	156 (95.7)	.003 (FE)
		Present	9 (18.8)	7 (4.3)	
Maternal response grade in ACA[g]	(173)	Grade 1	9 (25.0)	59 (43.1)	.048 (PCS)
		Grade 2	27 (75.0)	78 (56.9)	
Villitis	(215)	Absent	7 (14.0)	6 (3.6)	.014 (FE)
		Present	43 (86.0)	159 (96.4)	
Type if histiocytic villitis	(195)	Pattern A	24 (58.5)	135 (87.7)	<.001 (PCS)
		Pattern B	17 (41.5)	19 (12.3)	
Secondary pattern villitis	(215)	Absent	38 (76.0)	79 (47.9)	<.001 (PCS)
		Present	12 (24.0)	86 (52.1)	
Tertiary pattern of villitis	(215)	Absent	47 (94.0)	123 (74.6)	.003 (PCS)
		Present	3 (6.0)	42 (25.4)	
Stem villous villitis	(202)	Absent	8 (18.6)	7 (4.4)	.005 (FE)
		Present	35 (81.4)	152 (95.6)	
Proliferative vasculopathy	(215)	Absent	34 (68.0)	69 (41.8)	.001 (PCS)
		Present	16 (32.0)	96 (58.2)	
IUFD[h] severity	(119)	Mild-moderate	7 (24.1)	48 (53.3)	.006 (PCS)
		Severe	22 (75.9)	42 (46.7)	

[a]Confidence interval.
[b]Immunohistochemistry.
[c]Pearson's chi-squared.
[d]Antiretroviral treatment.
[e]Fisher's exact.
[f]Benzathine benzylpenicillin.
[g]Acute chorioamnionitis.
[h]Intrauterine fetal death.

serology and IHC tested positive for T. pallidum DNA on qPCR. Although not statistically significant, lower frequencies of positivity were observed in cases with IUFD changes ($p = .055$). The significant associations are summarized in Table 8.

Discussion

The serological diagnosis of congenital syphilis in neonates is hindered by the transplacental transfer of maternal antibodies and the high number of asymptomatic infants at birth. Placental examination plays a critical role in early diagnosis, treatment, and prevention of serious sequelae. The morphological features are not absolute and additional histological clues would be valuable in detecting placental syphilis. Our study evaluated the largest cohort of placentas diagnosed with syphilis to date, surpassing

Table 8. Treponema pallidum real-time PCR analysis.

Category	Variable	Result	95% CI[a]	
qPCR[b] diagnostic test analysis	Sensitivity	25.8%	15.5%	38.5%
	Specificity	75.0%	19.4%	99.4%
	Positive predictive value	94.1%	71.3%	99.9%
	Negative predictive value	6.1%	1.3%	16.9%

Category	(n)	Variable	Total (%)
qPCR result	(73)	Negative	56 (76.7)
		Positive	17 (23.3)

			qPCR result		
Category	(n)	Variable	Negative	Positive	p-value (test)
qPCR performed on	(73)	Cord	2 (3.6)	6 (35.3)	.002 (FE[e])
		Parenchyma	54 (96.4)	11 (64.7)	
IHC[d] result	(73)	Negative	43 (76.8)	7 (41.2)	.006 (PCS[c])
		Positive	13 (23.2)	10 (58.8)	
Necrotizing funisitis	(71)	Absent	43 (79.6)	8 (47.1)	.014 (FE)
		Present	11 (20.4)	9 (52.9)	
Maternal response in ACA[f]	(66)	Absent	5 (10.2)	7 (41.2)	.009 (FE)
		Present	44 (89.8)	10 (58.8)	
IUFD[g] change	(65)	Absent	15 (29.4)	8 (57.1)	.055 (PCS)
		Present	36 (70.6)	6 (42.9)	

[a] Confidence interval.
[b] Real-time polymerase chain reaction.
[c] Fisher's exact.
[d] Immunohistochemistry.
[e] Pearson's chi-squared.
[f] Acute chorioamnionitis.
[g] Intrauterine fetal death.

previous studies conducted between 1974 and 2022, which examined up to 51 infected placentas and 25 umbilical cords [18,21–26].

Routine examination

Placentomegaly is the only reported macroscopic abnormality in syphilitic placentas, observed in 12–100% of cases in previous studies and 56.4% in the current study when gestational age was known [18,21,26]. Whilst not specific to syphilis, placentomegaly can be the first finding to raise the index of suspicion for infection. The classical histological triad, considered highly suggestive of treponemal infection, has been reported in 11–43.3% of cases in larger studies and 100% in a study of three selected cases [21,23–26]. In the current cohort, the triad was present in only 28.4% of cases, limiting its diagnostic utility in daily practice. Additional morphological clues are needed to aid pathologists in considering the diagnosis of placental syphilis. Among the features of the histological triad, previous studies have reported the presence of villous immaturity, chronic and acute villitis, and proliferative vasculopathy in varying proportions of cases (38–100, 26.7–100, 5–73.3, and 68–100%, respectively) [18,21,23–26]. In the current study, chronic villitis (94%), and villous immaturity (65.6%) were among the three most common features. Acute villitis (37.7%) and proliferative vasculopathy (52.1%) were less prominent.

Acute chorioamnionitis (91.6%) was the second most common finding in this retrospective review, which had previously been reported in 31–68% of cases [18,21,23].

Previous studies have reported that assessment of the umbilical cord may provide clues, such as funisitis and necrotizing funisitis, although these findings are not commonly observed. Five studies found funisitis in 16–53.3% of cases and necrotizing funisitis in 5–27% of cases [18,21,22,24,25]. Schwartz et al. [22] also found that necrotizing funisitis was associated with umbilical cord thrombosis in 22% of cases and stillbirth in 78% of cases. In our study, non-necrotizing funisitis and necrotizing funisitis were infrequent, observed in only 7.6 and 26.1% of cases, respectively. Among cases with necrotizing funisitis, 91.5% were associated with stillbirth. Umbilical cord thrombosis was present in 1.4% of cases, and all cases were associated with necrotizing funisitis and stillbirth. Current grossing guidelines require only two sections of the umbilical cord to be submitted, leaving a considerable proportion of the cord unevaluated. Considering the focal nature of these lesions, evaluation of the entire cord would be necessary to determine the true frequency of necrotizing funisitis in placental syphilis.

In previous studies, other morphological features, such as erythroblastosis, deciduitis, avascular villi, and intervillous abscesses have been reported in varying frequencies, ranging from 12 to 48, 14 to 100, 39, and 20% of cases, respectively [18,21,23,25,26]. In our study, these features were observed in 42.8, 12.1, 37.7, and 9.3% of cases, respectively. Whilst most cases (81.5%) exhibited the classic description of enlarged immature villi with histiocytic villitis, 18.5% of cases demonstrated a subtle and previously undescribed pattern of histiocytic villitis (referred to as pattern B). This pattern was associated with lower frequencies of placentomegaly, villous immaturity, acute chorioamnionitis, additional patterns of villitis, intervillous abscesses, stem villous villitis, and proliferative vasculopathy. It was also less commonly diffuse in extent. This subtle pattern lacks many of the additional clues that would guide the pathologist to consider the diagnosis of placental syphilis, emphasizing the importance of meticulous microscopic examination of placental tissue.

Human immunodeficiency virus and treatment

The effect of HIV infection and treatment on the morphology of syphilis in placentas remains largely unexplored. In our cohort, HIV co-infection was observed in 22.2% of cases where HIV status was known. Previous studies have associated HIV infection with chorioamnionitis, plasma cell deciduitis, and decidual necrosis. A recent review highlighted low placental weight, maternal vascular malperfusion, acute chorioamnionitis, and lower frequencies of chronic villitis as associated placental features in HIV-positive individuals. Another study revealed that HIV-positive patients had lower frequencies of chorioamnionitis, but when present, it tended to be more severe. Patients undergoing ART exhibited lower frequencies of chorioamnionitis compared to untreated patients [12,31,36]. Our study found that HIV infection was associated with lower frequencies of necrotizing funisitis affecting all umbilical vessels, acute chorioamnionitis, and distal villous edema. Conversely, higher frequencies of pattern B histiocytic villitis, fibrin thrombi in the chorionic plate, fetal response in acute chorioamnionitis, and avascular villi were associated with HIV infection.

ART was found to be associated with higher frequencies of villitis and secondary patterns of villitis, proliferative vasculopathy, and higher grades of avascular villi. Conversely, treatment was linked to lower frequencies of deciduitis. These findings suggest that placental morphology in HIV-treated cases closely resembles that of HIV-negative patients, aligning with previous findings reported by Schuetz et al. in 2011 [31]. It can be hypothesized that HIV co-infection and the restoration of immune function through ART have an impact on the morphological features of placental syphilis. This information should be considered when available in these cases.

Benzathine benzylpenicillin treatment

Cases from mothers who received at least one dose of penicillin before delivery had lower frequencies of placentomegaly, villitis, and necrotizing funisitis involving all umbilical vessels. Among cases with acute chorioamnionitis, treated cases showed a higher frequency of fetal response but at a lower stage compared to untreated cases. Treatment was associated with higher frequencies of non-necrotizing funisitis, fibrin thrombi in the chorionic plate, and more severe IUFD changes. If the treatment status is not disclosed before placental examination, the lack of important morphological clues could potentially lead to a missed diagnosis.

Special investigations

Limited studies have explored the use of *Treponema pallidum* IHC and PCR in placental tissue. Most studies have focused on mucocutaneous lesions, with varying sensitivities and specificities reported. A review published in 2020 reported that IHC had a sensitivity of 49–92% and a specificity of 100%, whilst PCR for the *polA* gene had a sensitivity of 67% in FFPE tissue [37]. Polyclonal antibody IHC and various PCR methods targeting the *polA* gene in FFPE skin biopsies, showed positive IHC in 48.6–49% of cases and positive PCR in 38.9–100% of cases [14,29,30]. Buffet et al [16] used fresh frozen tissue and showed higher sensitivity with IHC (91%) and PCR (75%). The stage of disease was also shown to play a role in the detection of the organism [17,30]. IHC performed on FFPE placental tissue in 21 cases, demonstrated higher rates of positivity in the umbilical cord (42.9%) compared to the parenchyma (38.1%) [18]. HIV infection did not significantly affect the immunohistochemical detection of infective organisms in placental tissue [18]. PCR targeting the 47kd treponemal membrane antigen showed positivity in 17% of FFPE cases and higher positivity in cases with the diagnostic triad [23]. Nested PCR targeting the polA gene in fresh frozen placental tissue had positive results in 87% of parenchymal and 77% of umbilical cord sections [24]. Factors, such as antibiotic therapy, disease stage, sample type, length, and conditions of FFPE tissue storage, FFPE with cross-link formation leading to DNA degradation and inhibition of DNA amplification, can impact the performance of these tests [16–18,29,37].

Silver impregnation techniques (Dieterle, Steiner, or Warthin-Starry stains) are an alternative method to identify spirochetes in settings without access to IHC and PCR. Previous studies have reported rates of positivity of 50–69% in FFPE placental tissue,

most were in cases demonstrating the complete histological triad. Silver stains are not specific to *T. pallidum*, are technically difficult to perform and demonstrate extensive background staining [16,21,23,26,38].

Immunohistochemistry

This study evaluated the results of *T. pallidum* IHC in placental tissue. Positive IHC results were found in 89.3% of umbilical cord sections and 74.9% of parenchymal sections. When compared to serology, IHC had a sensitivity of 74.4% and confirmed the presence of the organism in 41 additional cases with negative or unknown serology. Lower frequencies of positivity were associated with ART, BPG treatment, non-necrotizing funisitis, pattern B histiocytic villitis, severe IUFD change, distal villous edema, and longer duration of FFPE block storage. Higher rates of positivity were observed in cases with villitis, secondary and tertiary patterns of villitis, stem villous villitis, proliferative vasculopathy, acute chorioamnionitis, and villous necrosis. These findings contradict previous reports that HIV does not affect the ability of IHC to detect the organism and lend further support to previous reports that antibiotic therapy and FFPE tissue preservation are associated with lower frequencies of positivity. Additionally, the use of DAB chromogen in IHC may be responsible for the refractile artifact found in some cases, and replacing DAB with a red chromogen can help avoid potential false-positive results.

Real-time polymerase chain reaction

qPCR targeting the *polA* gene was evaluated in FFPE tissue. Positive qPCR results were obtained in 75% of umbilical cord and 16.9% of parenchymal sections. When compared to serology, qPCR had a sensitivity of 25.8% and confirmed the diagnosis in one additional case where serology and IHC were negative. Higher frequencies of positivity were observed in cases with necrotizing funisitis, acute chorioamnionitis with fetal response, and villous necrosis. Lower frequencies of positivity were observed in cases with IUFD change and proliferative vasculopathy. HIV co-infection, ART, BPG treatment, and the length of FFPE block storage did not significantly impact the results.

Conclusions

In conclusion, our study revealed that villitis, acute chorioamnionitis, and villous immaturity were the most common findings in cases of placental syphilis. Placentomegaly, acute villitis, and proliferative vasculopathy were also helpful indicators. The classical histological diagnostic triad had limited diagnostic utility in routine practice. We identified two distinct patterns of histiocytic villitis, including a subtle pattern that may easily be overlooked without careful examination.

HIV co-infection, ART, and BPG treatment were shown to impact the morphological features and could potentially lead to a false-negative diagnosis. Accurate clinical information regarding HIV status, ART, and BPG treatment is important.

IHC and qPCR demonstrated sensitivities of 74.4 and 25.8%, respectively. These specialized investigations confirmed the presence of *T. pallidum* in 42 (19.5%) cases

with unknown or negative serology, proving to be valuable tools in the diagnostic arsenal of pathologists. IHC and qPCR had higher rates of positivity in umbilical cords with necrotizing funisitis. This distinctive focal lesion, prone to oversight under the current grossing guidelines, underscores the potential value of assessing the entire cord for improved case identification.

While established clinical indications for placental evaluation detect most cases, features, such as maternal HIV and placentomegaly may aid in identifying addi tional cases.

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Author contributions

Y.A.M., P.T.S., and D.M. conceived and designed the study. Y.A.M. and P.T.S. analyzed and inter preted the data. Y.A.M. dra ed the paper, and P.T.S., D.M., C.A.W., A.B., C.R.S., H.S.E., J.K., and A.J.G. critically revised it for intellectual content and provided nal approval. All authors take responsibility for all aspects of the work.

Disclosure statement

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Data availability statement

e corresponding author, Y.A.M., can provide the data supporting the ndings of this study upon reasonable request.

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