



eISSN 2284-0230 - pISSN 1826-883

<https://www.pagepressjournals.org/index.php/jbr/index>

Publisher's Disclaimer. E-publishing ahead of print is increasingly important for the rapid dissemination of science. The **Early Access** service lets users access peer-reviewed articles well before print / regular issue publication, significantly reducing the time it takes for critical findings to reach the research community.

These articles are searchable and citable by their DOI (Digital Object Identifier).

The **Journal of Biological Research** is, therefore, e-publishing PDF files of an early version of manuscripts that undergone a regular peer review and have been accepted for publication, but have not been through the typesetting, pagination and proofreading processes, which may lead to differences between this version and the final one.

The final version of the manuscript will then appear on a regular issue of the journal.

E-publishing of this PDF file has been approved by the authors.

J Biol Res 2024 [Online ahead of print]

To cite this Article:

Zaidi H, Lahlou L, Chraybi M, et al. **Correlation between placental bacterial PCR results and histological chorioamnionitis: a prospective study on 41 placentas.** *J Biol Res* doi: 10.4081/jbr.2024.11700

 ©The Author(s), 2024

Licensee [PAGEPress](#), Italy

Note: The publisher is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries should be directed to the corresponding author for the article.

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article or claim that may be made by its manufacturer is not guaranteed or endorsed by the publisher.

Correlation between placental bacterial PCR results and histological chorioamnionitis: a prospective study on 41 placentas

Hanaa Zaidi,^{1,2} Laila Lahlou,^{1,3} Mariem Chraybi,^{4,5} Amina Barkat,^{1,6} Sabah Elamrani,^{1,7} Najat Lamalmi^{1,2}

¹Faculty of Medicine and Pharmacy, Mohammed V University, Rabat; ²Laboratory of Pathological Anatomy and Cytology, Children's Hospital, CHU Ibn Sina, Rabat; ³Laboratory of Biostatistics and Clinical Research, Faculty of Medicine and Pharmacy, Rabat; ⁴Faculty of Medicine and Pharmacy, Abdelmalek Essaadi University, Tanger; ⁵Laboratory of Pathological Anatomy and Cytology, CHU Mohammed IV, Tanger; ⁶Mother and Child Health and Nutrition Research Team, Neonatology Department P5, Children's Hospital, CHU Ibn Sina, Rabat; ⁷Maternity Souissi, CHU Ibn Sina, Rabat, Morocco

Correspondence: Hanaa Zaidi, Mohammed V University, Faculty of Medicine and Pharmacy, 10170, Rabat, Morocco.

Tel.: +212667149040

E-mail: hanaa.zaidi@gmail.com

Key words: placenta, clinical chorioamnionitis, histological chorioamnionitis, polymerase chain reaction, bacterial placental infection.

Contributions: HZ: conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; wrote the paper; NL: conceived and designed the experiments; analyzed and interpreted the data; LL: analyzed and interpreted the data; AB, SA, and MC: contributed reagents, materials, analysis tools, or data.

Funding: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest: the authors declare no conflict of interest.

Ethics approval and consent to participate: our study exclusively utilized anonymized data. Per established regulations and guidelines in our country, ethical approval and informed consent were not deemed necessary for this particular study due to the anonymous nature of the data and the classification of the placenta as hospital waste.

Data availability: data will be made available on request.

Acknowledgments: the authors thank the Medical and paramedical personnel of the Laboratory of Pathological Anatomy and Cytology, Children's Hospital, CHU Ibn Sina, Rabat, Morocco; Medical and paramedical personnel of Maternity Souissi, CHU Ibn Sina, Rabat, Morocco and personnel of Biology and Medical Research Unit (BMRU) of the National Center for Energy Nuclear Sciences and Techniques (NCENST) for participation in the study.

Abstract

Chorioamnionitis or intra-uterine inflammation is considered the most common infection diagnosed in labor and delivery units worldwide. It is a leading cause of maternal morbidity and mortality, as well as neonatal death and sepsis. The heterogeneity of this clinical syndrome has been recently reported. The objectives of this study were to determine: 1) placental microbiology using molecular microbiological techniques; 2) diagnostic accuracy of the clinical criteria used to identify patients with placental bacterial infection; 3) relationship between placental bacterial infection and histological chorioamnionitis. This prospective cross-sectional study included 41 women diagnosed with clinical and histological chorioamnionitis. The presence of microorganisms in the placenta was determined by conducting placental analysis using a broad range of polymerase chain reactions (PCR). Bacterial placental infection (defined as the presence of the 16S gene detected in placental tissue using the molecular technique PCR) was observed in 63% (26/41) of parturients diagnosed with histological chorioamnionitis. The traditional criteria for diagnosing clinical chorioamnionitis exhibit poor diagnostic performance in accurately identifying proven intra-amniotic infection. The molecular analysis (PCR) of the placenta has suggested that acute chorioamnionitis commonly has a bacterial origin.

Introduction

Chorioamnionitis also referred to as "Inflammation or Intrauterine Infection" (triple I), represents the most prevalent pathology during pregnancy. It is characterized by acute inflammation of the amniotic membranes, chorionic plaque, and frequently the umbilical cord, often originating from bacterial sources. This condition is associated with a perinatal mortality rate of 12%, early neonatal mortality of 9%,¹ and in some instances, maternal mortality (less than 1%).² It most commonly arises in cases of premature rupture of the fetal membranes (75%) but can also occur with intact membranes (30%).¹⁻⁵

Chorioamnionitis is believed to stem from the ascending microbial invasion of the amniotic cavity (MIAC).⁶ This invasion prompts a maternal inflammatory response marked by clinical indicators such as fever, maternal tachycardia, uterine tenderness, malodorous discharge, and maternal leukocytosis.⁷⁻¹² Often, fetal tachycardia is present, which may reflect elevated maternal temperature and/or a fetal inflammatory response.¹³⁻¹⁶

In 2021, a study by Romero *et al.*¹⁷ unveiled that clinical chorioamnionitis is not a singular entity, but rather a syndrome associated with i) proven intra-amniotic infection, ii) sterile intra-amniotic inflammation, or iii) maternal signs of systemic inflammation without intra-amniotic inflammation. The most detected microorganisms in the amniotic fluid of term patients with clinical chorioamnionitis were *Ureaplasma* species and *Gardnerella vaginalis*.¹⁸

Consequently, the objectives of this study were to ascertain: i) the placental microbiology through the utilization of molecular techniques (polymerase chain reactions, PCR); ii) the diagnostic precision of the clinical criteria used for identifying patients with intra-amniotic infection; and iii) the relationship between placental bacterial infection and histological chorioamnionitis.

Materials and Methods

Study population

This prospective study examined 41 placentas collected from the Maternity of Ibn Sina Hospital of Rabat, Morocco. Demographic and clinical data were recorded at the time of patient admission and compiled in an SPSS 13.0 database. To ensure data confidentiality, each case was assigned an anonymous number.

Patients included in the study were those admitted to Souissi Maternity Hospital in Rabat between February 2018 and October 2019. Inclusion criteria encompassed suspicion of maternal or neonatal infection with the presence of one or more of the following signs: i) Prematurity (<37 weeks gestational age), ii) Premature rupture of membranes (PROM) exceeding 12 hours, maternal fever surpassing 38°C, and iii) meconium-stained amniotic fluid. Exclusion criteria encompassed twin pregnancies and abnormal placentation (placenta accreta, percreta, and previa).

Clinical definitions

Clinical chorioamnionitis was diagnosed based on the presence of an elevated maternal temperature (cut-off $\geq 37.8^{\circ}\text{C}$) associated with two or more of the following criteria: i) maternal tachycardia (heart rate > 100 beats/min); ii) fetal tachycardia (heart rate > 160 beats/min); iii) uterine tenderness; iv) malodorous vaginal discharge; and v) maternal leukocytosis (leukocyte count $> 15,000$ cells/mm³).^{19,20}

The criteria for an elevated maternal temperature were the same as those proposed by Gibbs and Duff⁷ and Gibbs *et al.*⁸ and subsequently employed by other investigators studying intra-amniotic infection.²¹⁻²⁴

Sample collection

Molecular identification of infection was carried out at the Biology and Medical Research Unit (BMRU) of the National Center for Energy, Nuclear Sciences, and Technologies in Rabat.

The analysis focused on 41 placental samples collected from parturients who exhibited signs of clinical chorioamnionitis. Among these cases, 18 placental tissues (chorion and chorionic villus) were frozen at -80°C , and 23 were fixed in paraffin blocks (amniotic membrane blocks and placental tissue blocks).

Fresh tissues were directly used for DNA extraction, while for paraffin-embedded tissues, a deparaffinization step was necessary before proceeding with the extraction of nucleic acids.

Placental histopathologic examination

Placentas were collected at the Souissi Maternity of Ibn Sina Hospital in Rabat and then transferred to the Laboratory of Anatomy and Pathological Cytology at the Children's Hospital, CHU Ibn Sina in Rabat.

Sampling of the placentas was conducted following the protocols established by the Perinatology Research Directorate, as previously described in our article published in 2020.²⁵

For each case, a minimum of five full-thickness sections of the chorionic plate, three sections of the umbilical cord, and three rolls of chorioamniotic membrane were examined by pathologists.

Acute inflammatory lesions of the placenta (maternal inflammatory response and fetal inflammatory response) were diagnosed according to the Amsterdam consensus (Table 1).^{26,27}

Detection of bacterial infection utilizing molecular methods (PCR)

Tissue dewaxing

Each of the 23 paraffin blocks was meticulously cut using a microtome. In total, five sections of 5 µm were produced for each sample, with care taken not to deplete the paraffin block.

These sections were gently placed in labeled Eppendorf tubes and stored at +4°C.

After each section, it is essential to clean the microtome, blade, and forceps thoroughly using xylene and ethanol to prevent any potential cross-contamination.

For the deparaffinization process, a volume of 700 µL to 1 mL of xylene was added to the tube containing the sections. The mixture was then vortexed and incubated at 50°C for 20 minutes. After centrifugation at 10,000 rpm for 5 minutes, the supernatant was discarded, and the pellet was washed a second time with xylene to remove any remaining paraffin residue.

To eliminate traces of xylene, approximately 1 mL of absolute ethanol was added to the Eppendorf tube containing the tissue. The tube was shaken up and down. After centrifugation at 12,000 rpm for 5 minutes, the ethanol was removed, and the samples were dried either under vacuum or at 37°C until the ethanol had completely evaporated.²⁸⁻³² In terms of the methodologies employed in our work, we drew inspiration from several previous studies that utilized similar approaches. Additionally, we consulted established protocols in the academic

literature to develop our experimental protocols. These protocols were adapted to our specific context, taking into account the particularities of our study. While our protocols were influenced by previous work, we also conducted specific adjustments and validations to meet the needs of our research, making them partly a modification of existing protocols, but with significant adaptations for our particular study.

DNA extraction

DNA extraction was conducted following the conventional alkaline lysis method and purification using phenol chloroform. Initially, the pellet was suspended in 600 μL of lysis buffer containing proteinase K (final concentration of 200 $\mu\text{g}/\mu\text{L}$). The volume could be adjusted based on the pellet quantity. The mixture was vortexed and then incubated overnight at 37°C with gentle agitation. If the pellet was not fully digested, this step could be repeated. After digestion, the tubes were incubated at 90°C for 5 minutes to inactivate proteinase K.

The cell lysate was then combined with phenol-chloroform in equal volumes, and the resulting mixture was vortexed for 1 minute until an emulsion was formed. After centrifugation for 5 minutes at 13,000 rpm, two phases were separated: an organic phase at the bottom and an aqueous phase at the top containing the extracted DNA, which was separated from a protein layer. The aqueous phase was carefully collected without disturbing the protein layer and transferred to a new tube containing 150 μL of 7.5M ammonium acetate. To this mixture, 2 volumes of absolute ethanol were added, and the mixture was inverted to promote DNA precipitation. After centrifugation at 13,000 rpm for 5 minutes, the ethanol was removed, and the DNA pellet was washed with 70% ethanol, dried, and then resuspended in 20 μL of sterile distilled water (DNase-free). The extracted DNA was either used immediately or stored at -20°C.^{28,32}

DNA assay

To assess the quality of extraction and determine the DNA concentration ($\text{ng}/\mu\text{L}$) in each sample, the specimens were analyzed using a NanoDrop 8000 Spectrophotometer® (Thermo Scientific, Boston Massachusetts, USA) coupled with the ND8000 V2.0.0 software, enabling the assessment of optical densities at 260 and 280 nm.

The measurement at 260 nm provides the means to evaluate DNA concentration, while the OD260/OD280 ratio serves to gauge DNA purity.

Furthermore, the extracted DNA is deemed "pure" when the ratio ranges between 1.8 and 2. A notably lower ratio (<1.8) indicates the presence of proteins, phenol, or other contaminants that absorb at or near 280 nm. In such cases, extraction and/or re-purification procedures were repeated to enhance the quality of the extracted DNA.^{28,32}

Qualitative amplification by β -globin

The integrity of the DNA extracts was assessed through PCR amplification of a 268 base pair (bp) sequence from the gene coding for β -globin using PC04 and GH20 primers (Table 2).

The reaction was conducted in a final volume of 25 μ L, following the composition outlined in Table 3. For each PCR run, it is essential to include a negative control that lacks template DNA to verify potential reagent contamination. In the negative control, sterile water replaces the DNA. Extracted DNA was deemed of good quality if a visible amplification of the β -globin fragment was achieved, and it was of the correct size.

The reaction commences with a denaturation step at 94°C for 5 minutes. Following this DNA template denaturation, 40 amplification cycles are conducted, each consisting of denaturation at 94°C for 1 minute, primer hybridization at 55°C for 1 minute, and elongation at 72°C for 1 minute. After the final cycle, an additional elongation at 72°C for 1 minute is carried out to ensure the completion of elongation for all newly synthesized DNA strands. PCR products are subsequently incubated at 4°C until utilized. All PCR amplifications were performed in a GeneAmpR PCR System 9700 thermocycler (AB/Applied Biosystems, Foster City, CA).^{28,32}

Bacterial identification by 16S rDNA broad-spectrum

The purpose of this technique is to amplify the 16S ribosomal RNA gene present in all bacteria. Amplification of the 16S gene was performed using the 16Sa (forward) and 16Sb (reverse) primers, enabling the amplification of a fragment approximately 1,500 bp in length (Table 4).

The composition of the 16S PCR reaction mixture is detailed in Table 5. For each series of PCR, it is essential to include a negative control without template DNA to ensure the absence of potential reagent contamination; in this control, sterile water replaces the DNA. Additionally, a positive control containing bacterial DNA extracted from *Streptococcus* B is included. PCR was conducted using the GeneAmpR PCR System 9700 thermocycler (AB/Applied Biosystems, Foster City, CA), following these conditions: after an initial denaturation at 94°C for 5 minutes, 40 cycles were performed. Each cycle consisted of denaturation at 94°C for 1 minute, hybridization at 59°C for 1 minute and extension at 72°C for 30 seconds. After the final

cycle, an extra extension at 72°C for 10 minutes was carried out. The PCR products were either used immediately or stored at -20°C until needed.^{28,32}

Analysis of PCR products

After PCR amplification, electrophoresis was conducted each time to visualize the obtained amplicons. The PCR products were separated through electrophoresis on a 2% agarose gel containing BET. The agarose gel was laid out horizontally, and a volume of 7 µL of the amplified PCR product from each sample was mixed with 3 µL of loading buffer. This mixture was then placed into the wells of the gel, which was submerged in an electrophoresis tank containing 0.5X TBE buffer. Additionally, 4 µL of the molecular weight marker (Hyper-Ladder™ IV, Bioline, ranging from 100 bp to 1013 bp) was deposited to aid in assessing the size of the amplicons.

Electrophoresis was carried out for one hour at 100 volts in an electrophoresis tank connected to an electric generator (Bio-Rad Laboratories, Marnes-la-Coquette, France).

DNA was visualized under ultraviolet illumination (302 nm). The amplified fragment appeared as a band, the length of which was compared to the various fragments of the size marker.^{28,32}

Statistical analysis

Clinical, histopathological, and molecular characteristics were assessed using the statistical software SPSS version 13.0 (Licensed by University Mohammed V-Rabat). The level of statistical significance was set at $p < 0.05$.

Both descriptive and analytical analyses were conducted, including univariate and multivariate approaches. For the descriptive study, variables exhibiting a Gaussian distribution were presented as mean and standard deviation, and their comparison was carried out using Student's t-test. Variables with a non-Gaussian distribution were represented as median and interquartile ranges and compared using the Mann-Whitney test.

Qualitative variables were expressed in terms of numbers and percentages and subsequently compared using the exact Chi-square and Fisher tests based on the specific conditions for their application. The search for a correlation between placental infection and histological chorioamnionitis was performed through logistic regression in both uni- and multivariate analyses. Variables with a significant p-value were included in a multivariate model to explore their association with histological chorioamnionitis.

Ethics approval and consent to participate

Our study was conducted according to the principles of the Declaration of Helsinki. Ensuring the confidentiality and protection of participants' personal information was of utmost importance during data collection and analysis. Each case was assigned an anonymous number to ensure that no personal data was identifiable.

As all analyses were performed using anonymized data, ethical approval, and written informed consent were not required due to the anonymous nature of the data.

Results

Demographic and clinical data

The average age of the patients was 28 ± 6 years. The gestational age averaged 39 ± 2 weeks. Cesarean sections were performed in 27% of the population. Experience with one or more induced abortions due to infection, in-utero fetal death, stillbirth, and other medical conditions was observed in only eight patients. Clinical signs of infection, such as PROM, fever, and stained amniotic fluid, were present in 43%, 21.6%, and 17.8% of the population, respectively.

Results of molecular examination

Microbial infection in placental tissue and amniotic membranes of 41 parturients presenting signs of presumed chorioamnionitis was investigated by amplifying the 16S gene specific to all bacteria using conventional PCR. The results revealed that 63% of the cases (26/41) exhibited an amplification band of the expected size.

Correlation between molecular examination and clinical parameters

To identify predictive clinical markers for microbial infection in amniotic membranes, we correlated PCR results with infection-related clinical signs, such as PROM, fever, and tinted amniotic fluid. The outcomes are detailed in Table 6. Statistical analysis demonstrated that no clinical signs (PROM, fever, and tinted amniotic fluid) displayed a significant correlation with bacterial infection.

Correlation between molecular and pathological examination

To determine the association between the presence of microbial DNA in placental tissues and amniotic membranes and histological chorioamnionitis, PCR results were cross-referenced

with the findings of histological examination of the placenta. The results are presented in Table 7. Statistical analysis did not indicate any significant correlation between bacterial infection and histological criteria.

Discussion

The advancement of molecular techniques for detecting bacterial DNA in placental tissues and amniotic membranes has illuminated the fact that conventional bacterial culture methods often underestimate the prevalence of bacteria in these tissues. This is because some microbes are challenging to culture or cannot be cultured at all.²⁸ Han *et al.* demonstrated that molecular techniques not only identify microbial invasion but also reveal the diversity of bacterial species involved.²⁹

The majority of molecular studies on infections indicate that bacterial prevalence in amniotic membranes is higher than in amniotic fluid.^{30,31} Therefore, exploring bacterial colonization in the amniotic membranes is deemed more reliable than in the amniotic fluid. Particularly for bacteria accessing the uterine cavity through the vagina, the membranes serve as the initial point of contact, and it is at the chorizo-deciduous interface where interaction with the innate immune system takes place.

However, not all studies using molecular techniques have reported high rates of bacterial colonization due to challenges in amplifying bacterial DNA in placental tissue.³² To address this, our study optimized the protocol by extracting bacterial DNA from two distinct sites for each placenta (amniotic membranes and placental tissue) to enhance the quality of DNA extraction.

Using this approach, we utilized universal 16S ribosomal DNA primers, capable of amplifying a DNA fragment from all known bacteria, to investigate bacterial colonization in amniotic membranes of 41 cases with presumed chorioamnionitis. We identified microbial invasion in amniotic membranes in 63% of cases (26/41), consistent with the findings reported by DiGiulio *et al.* (55%), who employed a similar molecular approach.³³

To ascertain the correlation between clinical signs suggestive of chorioamnionitis and bacterial colonization in the amniotic membranes, we compared PCR results with various clinical parameters. In our study, the association between rupture of membranes (RPM) and bacterial colonization in amniotic membranes and placental tissue was confirmed by a higher rate of

bacterial infection in the RPM group (22/26 cases) than in the intact membranes group (4/26 cases). This supports the observations of Cahill *et al.*,³⁴ further affirming that chorioamnionitis or intrauterine bacterial infection is more frequent after PROM. Bacterial invasion is facilitated by membrane rupture, making the passage of bacteria easier.

For the 4 cases that exhibited bacterial infection with intact membranes, several explanations are plausible: i) the presence of bacteria does not invariably trigger an inflammatory response or PROM. This could be attributed to brief bacterial colonization of the membranes that did not lead to an established infection; ii) universal treatment of PROM cases with erythromycin (but not cases with intact membranes) could have reduced bacterial DNA load below detection limits in certain instances, thereby underestimating the role of bacteria in this group. However, a study by Gomez *et al.* indicated that administering antibiotics to women with PROM did not alter intraamniotic infection rates.³⁵

Our study indicated that bacterial colonization in amniotic membranes and placental tissues is more prevalent in parturients who gave birth vaginally. This finding aligns with Seong *et al.*,³⁶ confirming that the prevalence of bacteria in women giving birth vaginally, preterm, or at term, is higher compared to those undergoing cesarean section. This suggests that the process of labor and vaginal birth heightens bacterial prevalence, which could occur through vaginal flora contamination or because of labor itself.

Furthermore, our study demonstrates that the presence of bacteria in amniotic membranes in women giving birth at term does not necessarily result in preterm birth. This concurs with Steel *et al.*,³⁷ although Jones *et al.*³⁸ argued that bacteria play a significant role in many cases of preterm birth.

Several studies have highlighted a robust association between clinical parameters such as fever, stained amniotic fluid, neonatal morbidity, and microbial invasion in amniotic membranes, confirming a dose-response relationship and a causal link between clinical parameters and bacterial infection.³⁹⁻⁴² However, our results differ from these studies due to the small sample size.

Numerous studies have established a correlation between histological chorioamnionitis, characterized by the presence of neutrophils in amniotic membranes, and the detection of bacteria

using molecular techniques (PCR). These studies have indicated a high degree of concordance between the two examinations.^{29,32,43}

In our study, 69% of histological chorioamnionitis cases exhibited bacterial presence in their amniotic membranes, as detected by the 16S gene through PCR. In contrast, only 31% of cases without bacteria in their amniotic membranes had histological chorioamnionitis. Conversely, the 31% of histological chorioamnionitis cases that yielded negative PCR results suggest that histological chorioamnionitis is not always linked to infection, possibly reflecting "sterile" inflammation due to extraplacental causes, as noted in several studies.^{18,44}

Finally, several factors could influence our objective of investigating the correlation between bacterial presence in placental tissues and amniotic membranes and histological chorioamnionitis, as well as certain clinical signs of infection. These factors include the small size of our sample, rendering our molecular results statistically insignificant ($p > 0.05$). Additionally, bacteria detected in specimens from vaginal deliveries may indicate tissue contamination during passage through the vagina rather than bacterial colonization before delivery.^{45,46}

Conclusions

In this study, we examined 41 placentas from parturients with clinical signs of chorioamnionitis using PCR technology, aiming to establish a correlation between placental bacterial PCR results and the clinical and histological aspects of chorioamnionitis.

Our study demonstrated the presence of the 16S gene sequence in 63% of chorioamnionitis cases. Additionally, we observed a correlation between the presence of microbial DNA in placental tissues and the clinical and histological parameters of chorioamnionitis.

References

1. Redline RW. Placental inflammation. *Semin Neonatol* 2004;9:265-74.
2. Report of the National Committee of Experts on Maternal Mortality. INSERM, December 2006.
3. Baergen RN. The placenta as witness. *Clinics Perinatol* 2007;34:393-407.

4. Kim CJ, Romero R, Chaemsaithong P, et al. Acute chorioamnionitis and funisitis: definition, pathologic features, and clinical significance. *Am J Obstet Gynecol* 2015;213:S29-S52.
5. Cornette L. Fetal and neonatal inflammatory response and adverse outcome. *Semin Fetal Neonat Med* 2004;9:459-70.
6. Romero R, Gomez-Lopez N, Winters AD, et al. Evidence that intra-amniotic infections are often the result of an ascending invasion - a molecular microbiological study. *J Perinat Med* 2019;47:915-31
7. Gibbs RS, Duff P. Progress in pathogenesis and management of clinical intraamniotic infection. *Am J Obstet Gynecol* 1991;164:1317-26.
8. Gibbs RS, Blanco JD, St Clair PJ, et al. Quantitative bacteriology of amniotic fluid from women with clinical intraamniotic infection at term. *J Infect Dis* 1982;145:1-8.
9. Gilstrap LC 3rd, Cox SM. Acute chorioamnionitis. *Obstet Gynecol Clin North Am* 1989;16:373-9.
10. Willi MJ, Winkler M, Fischer DC, et al. Chorioamnionitis: elevated interleukin-6 and interleukin-8 concentrations in the lower uterine segment. *J Perinat Med* 2002;30:292-6.
11. Lee SE, Romero R, Kim CJ, et al. Funisitis in term pregnancy is associated with microbial invasion of the amniotic cavity and intra-amniotic inflammation. *J Matern Fetal Neonatal Med* 2006;19:693-7.
12. Redline RW. Inflammatory response in acute chorioamnionitis. *Semin Fetal Neonatal Med* 2012;17:20-5.
13. Schiano MA, Hauth JC, Gilstrap LC III. Second-stage fetal tachycardia and neonatal infection. *Am J Obstet Gynecol* 1984;148:779-81.
14. Coulter J, Turner M. Maternal fever in term labour in relation to fetal tachycardia, cord artery acidemia and neonatal infection. *Br J ObstetGynaecol* 1998;105:242.
15. Chaiworapongsa T, Romero R, Kim JC, et al. Evidence for fetal involvement in the pathologic process of clinical chorioamnionitis. *Am J Obstet Gynecol* 2002;186:1178-82.
16. Buhimschi CS, Abdel-Razeq S, Cackovic M, et al. Fetal heart rate monitoring patterns in women with amniotic fluid proteomic profiles indicative of inflammation. *Am J Perinatol* 2008;25:359-72.
17. Romero R, Pacora P, Kusanovic JP, et al. Clinical chorioamnionitis at term X: microbiology, clinical signs, placental pathology, neonatal bacteremia, and implications for clinical care. *J Perinat Med* 2021;49:275-98

18. Romero R, Miranda J, Kusanovic JP, et al. Clinical chorioamnionitis at term I: microbiology of the amniotic cavity using cultivation and molecular techniques. *J Perinat Med* 2015;43:19–36.
19. Hauth JC, Gilstrap LC 3rd, Hankins GD, et al. Term maternal and neonatal complications of acute chorioamnionitis. *Obstet Gynecol* 1985;66:59–62.
20. Gibbs RS, Dinsmoor MJ, Newton ER, et al. A randomized trial of intrapartum versus immediate postpartum treatment of women with intra-amniotic infection. *Obstet Gynecol* 1988;72:823–8.
21. Newton ER. Chorioamnionitis and intraamniotic infection. *Clin Obstet Gynecol* 1993;36:795–8.
22. Soper DE, Mayhall CG, Dalton HP. Risk factors for intraamniotic infection: a prospective epidemiologic study. *Am J Obstet Gynecol* 1989;161:562–6; discussion 6-8.
23. Newton ER, Prihoda TJ, Gibbs RS. Logistic regression analysis of risk factors for intra-amniotic infection. *Obstet Gynecol* 1989;73:571–5.
24. Curtin WM, Katzman PJ, Florescue H, et al. Accuracy of signs of clinical chorioamnionitis in the term parturient. *J Perinatol* 2013;33:422–8.
25. Zaidi H, Lamalmi N, Lahlou L, et al. Clinical predictive factors of histological chorioamnionitis: case-control study. *Heliyon* 2020;6:e05698.
26. Khong TY, Eoghan EM, Ilana A, et al. Sampling and definitions of placental lesions: Amsterdam Placental Workshop Group Consensus Statement. *Arch Pathol Lab Med* 2016;140:698-713.
27. Khong TY, Mooney EE, Nikkels PGJ, et al. *Pathology of the Placenta. A practical guide.* Springer Cham; 2019.
28. Gardella C, Riley DE, Hitti J, et al. Identification and sequencing of bacterial rDNAs in culture-negative amniotic fluid from women in premature labour. *Am J Perinatol* 2004;21:319-23.
29. Han YW, Shen T, Chung P, et al. Uncultivated bacteria as etiologic agents of intra-amniotic inflammation leading to preterm birth. *J Clin Micro* 2009;47:38-47.
30. Romero R, Quintero R, Oyarzun E, et al. Intraamniotic infection and the onset of labor in preterm premature rupture of the membranes. *Am J Obstet Gynecol* 1988;159:661-6.
31. Gauthier DW, Meyer WJ, Bieniarz A. Correlation of amniotic fluid glucose concentration and intraamniotic infection in patients with preterm labor or premature rupture of membranes. *Am J Obstet Gynecol* 1991;165:1105-10.

32. Onderdonk AB, Delaney ML, DuBois AM, et al. Detection of bacteria in placental tissues obtained from extremely low gestational age neonates. *Am J Obstet Gynecol* 2008;198:110.e1-110.e7.
33. DiGiulio DB, Romero R, Kusanovic JP, et al. Prevalence and diversity of microbes in the amniotic fluid, the fetal inflammatory response, and pregnancy outcome in women with preterm pre-labor rupture of membranes. *Am J Reprod Immunol* 2010;64:38-57.
34. Cahill RJ, Dougan G, O'Gaora P, et al. Universal DNA primers amplify bacterial DNA from human fetal membranes and link *Fusobacterium nucleatum* with prolonged preterm membrane rupture. *Mol Hum Reprod* 2005;11:761-65.
35. Gomez R, Romero R, Nien JK, et al. Antibiotic administration to patients with preterm premature rupture of membranes does not eradicate intra amniotic infection. *J Maternal-Fetal Neo Med* 2007;20:167-73.
36. Seong HS, Lee SE, Kang JH, et al. The frequency of microbial invasion of the amniotic cavity and histological chorioamnionitis in women at term with intact membranes in the presence or absence of labor. *Am J Obstet Gynecol* 2008;199:375.e1-375.e5.
37. Steel JH, Malatos S, Kennea N, et al. Bacteria and inflammatory cells in fetal membranes do not always cause preterm labour. *Ped Res* 2005;57:404-11.
38. Jones HE, Harris KA, Azizia M, et al. Differing prevalence and diversity of bacterial species in fetal membranes from very preterm and term labor. *PLoS ONE* 2009;4:e8205.
39. DiGiulio DB, Romero R, Amogan HP, et al. Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. *PLoS ONE* 2008;3:e3056.
40. Hill AB. The environment and disease: association or causation? *Proc R Soc Med* 1965;58:295-300.
41. Fredericks DN, Relman DA. Sequence-based identification of microbial pathogens: a re-consideration of Koch's postulates. *Clin Microbiol Rev* 1996;9:18-33.
42. Romero R, Gomez R, Chaiworapongsa T, et al. The role of infection in preterm labour and delivery. *Paediatr Perinat Epidemiol* 2001;15:41-56.
43. De Felice C, Toti P, Santopietro R, et al. Small thymus in very low birth weight infants born to mothers with subclinical chorioamnionitis. *J Pediatr* 1999;135:384-86.
44. Romero R, Jezid M, Tinnakorn C, et al. A novel molecular microbiologic technique for the rapid diagnosis of microbial invasion of the amniotic cavity and intra-amniotic infection in preterm labor with intact membranes. *Am J Reprod Immunol* 2014;71:330-58.

45. Boutaga K, van Winkelhoff AJ, Vandenbroucke-Grauls CM, et al. Periodontal pathogens: A quantitative comparison of anaerobic culture and real time PCR. *FEMS Immunol Med Micro* 2005;45:191-9.
46. Fredricks DN, Fiedler TL, Thomas KK, et al. Targeted PCR detection of vaginal bacteria associated with bacterial vaginosis. *J Clin Micro* 2007;45:3270-76.

Table 1. Maternal inflammatory response.

Stage 1. Subchorionitis/Acute chorionitis	Grade 1. Non-severe
Stage 2. Acute chorioamnionitis	Grade 2. Severe (leukocytes consisting of confluent neutrophilic polymorphonuclear cells with subchorionic micro-abscesses)
Stage 3. Necrotizing chorioamnionitis	

Table 2. Primers used for the amplification of a β -globin gene sequence.

Gene	Primers	Sequences	Size
β -globin	PC04	5' -CAACTTCATCCACGTTTCACC -3'	268pb
	GH20	5' -GAAGAGCCAAGGACAGGTAC -3'	

Table 3. β -Globin PCR reaction mix volumes and concentrations.

Elements	Volume(μ L)	Final concentration
Water	16.875	-
PCR Buffer 10X	5	2X
MgCl ₂	0.75	2.5 mM
dNTP	2	200 μ M
PrimersPC05	0.5	0.4 μ M
Primers GH20	0.5	0.4 μ M
Taq DNA polymerase (Promega, France)	0.125	units
DNA (50ng/ μ L)	2	4ng/ μ L

Table 4. Primers used for the amplification of a 16S gene sequence.

Gene	Primers	Sequences	Size
16S rRNA	16Sa	5'-AGAGTTTGATCCTGGCTCAG-3'	1541pb
	16Sb	5'-AAGGAGGTGATCCAGCCGCA-3'	

Table 5. 16S PCR reaction mix volumes and concentrations.

Elements	Volume (μ L)	Final concentration
Water	16.875	-

PCR Buffer 10X	5	2X
Primers F	0.5	0.4μM
Primers R	0.5	0.4μM
Taq DNA polymerase (Promega, France)	0.125	units
DNA (50ng/μL)	2	4ng/μL

Table 6. Clinical characteristics of our patients according to PCR results.

	PCR Positive	PCR Negative	
Variable	N=26 (%)	N=15 (%)	p value
Fever (°C)			0.091
Yes	7 (46.7)	8 (53.3)	
No	19 (73.0)	7 (27.0)	
Mode of delivery			0.197
Vaginal	15 (75.0)	5 (25)	
Cesarean	11 (52.4)	10 (47.6)	
Premature rupture of membranes			1
Yes	22 (62.9)	13 (37.1)	
NO	4 (66.7)	2 (33.3)	
Amniotic fluid			0.7
Stained	9 (69.2)	4 (30.8)	
Normal	17 (60.7)	11 (39.3)	
Gestational age (weeks)	39 ± 1.1*	39 ± 1.3	0.845

* Means ± SD.

significant ($p < 0.001$).

Table 7. Histological characteristics of our patients according to the results of the PCR.

Histological chorioamnionitis	PCR Positive	PCR Negative
	N=26 (%)	N=15 (%)
Yes	20 (69)	9 (31)
NO	6 (50)	6 (50)

Submitted: 31 August 2023

Accepted: 21 February 2024

Early access: 18 April 2024