Medicine

# INTEROBSERVER VARIABILITY IN PLACENTAL HISTOPATHOLOGY DIAGNOSIS OF INFLAMMATORY LESIONS: A STATISTICAL EXPERIMENT

**Zamfir-Radu IONESCU[1]**
**Georgiana-Cristina IONESCU[2]**
**Diana-Ioana VOICU[3]**
**Maria SAJIN[4]**

**ABSTRACT:**
*THE WORLDWIDE TREND FOR MEDICAL PRACTICE, IN GENERAL, AND FOR HISTOPATHOLOGICAL LESIONS DIAGNOSIS, IN PARTICULAR, TURNS IN FAVOR FOR PROTOCOL USE, AS IN MANY COUNTRIES AND SPECIALTIES, THESE ARE NOT WELL IMPLEMENTED. OUR AIM REFERS TO AN EXPERIMENT THAT INVOLVED FIVE AVERAGE EXPERIENCED OBSERVERS THAT EXAMINED 10 PLACENTAS WITH CHORIOAMNIONITIS, 4 ACUTE CASES AND 6 CHRONIC CASES. THEY HAD TO ISSUE THE DEGREE OF EXTENT OF BASIC LESIONS: HYALINE DEPOSITION, INFLAMMATION, FIBROSIS, NECROSIS AND HYPEREMIA. THE INSTRUMENT USED WAS A LIKERT SCALE WITH FIVE DEGREES OF INTENSITY. KAPPA STATISTICAL CALCULATIONS PROVED A SLIGHT DIFFERENCE BETWEEN THE FIVE EXAMINATORS IN REGARDING THE APPRECIATION OF LESION SEVERITY. THE INTEROBSERVER AGREMEENT WAS BETWEEN 0.04 TO 0.14 WITH HIGHER CONSISTENCY TOWARD OBVIOUS LESIONS WITH ALPHA VALUES FROM 0.61 TO 0.81.THUS SHOWING THE NECESSITY FOR A FAR PRECISE DIAGNOSIS PROTOCOL TO BE IMPLEMENTED, AS THE LESIONS IN CHORIOAMNIONITIS PLACENTA MA BE VERY DIFFICULT TO APRECIATE.*

**KEY WORDS:** INTEROBSERVER AGREEMENT, CHORIOAMNIONITIS, PLACENTA, INFLAMMATION, HISTOPATHOLOGY, DIAGNOSIS.

## INTRODUCTION

Chorioamnionitis (CHO) or amniotic infection is an inflammation of the membranes and chorion, produced due to ascending microbial bacterial flora in the setting of membrane

[1]PhD, Department of Pathology, Emergency University Hospital, Bucharest, Romania; e-mail: dr.raduionescu@yahoo.com.
[2]Department of Neonatology and Pediatrics, University Hospital, Bucharest, Romania; e-mail: oprea_22@yahoo.com.
[3] MD. Department of Obstetrics and Gynecology, Emergency University Hospital, Bucharest, Romania.
[4] M.D., PhD, Department of Pathology, The University Emergency Hospital, Bucharest, Romania; Head of Morphology and Pathology Department, The University of Medicine and Pharmacy, "Carol Davila", Bucharest; e-mail: maria_sajin@yahoo.com.

rupture. However, CHO may appear with intact membrane features as it may be produced bay infracellular agents like "Ureaplasma" species and "Mycoplasma hominis", incidentally discovered in lower genital tract at over 70% of women[5]. Between clinical and histologic CHO still exists a large gap, the latter being based on pathologic findings on microscopic examination of the placenta that might encompass clinically unapparent chorioamnionitis as well as clinically obvious CHO. The clinical signs are represented by the presence of fever, uterine tenderness, maternal or fetal tachycardia and purulent amniotic fluid, in the absence of other etiologies for these signs and symptoms[6]. Pregnant women consultation may reveal no sign or symptoms of infection or it may reveal clinical aspects that could lead to a misdiagnosis, like ill appearing female patient, even toxic, with hypotension, diaphoresis, cool or clammy skin[7]. Therefore, the accuracy of diagnosis in CHO, either acute or chronic, is not always reliable, as some studies show that fever have 61% accuracy, with high specificity an low sensitivity, and tachycardia with 52.4% accuracy with a very low sensitivity, thus histologic placental abnormalities consistent with CHO being a more frequent discovery with no prior specific symptomatology[8]. Blood count may reveal maternal leukocytosis (white blood cells>12000-15000 / $mm^3$) or the presence of a left shift or bandemia. Some studies proved high-levels of C-reactive protein together the presence of soluble intercellular adhesion molecule 1 (sICAM 1) and interleukin 6 in CHO, but at least some of these markers are far from being reliable to use on a routine basis[9]. Some authors suggest high increase in third day of newborns of WBC to be strongly correlated with CHO[10]. Histologic chorioamnionitis is defined by the existence of acute inflammatory changes on the microscopic examination of amniotic membranes and chorion in placenta and funisitis. This implies the presence of polymorphonuclear leukocytes per high power field, graded trough detailed systems of lesion classification, location, degeneration in order to estimate intensity and CHO evolution[11]. The pathologist and the gynecologist should grossly observe either a normal placenta or with dull, opaque membranes with yellow-green color and amniotic fluid that have opalescent aspect, even cloudy (figure 1, figure 2). Purulent exudate may be present. Microscopically, neutrophil infiltrate of membranes, funisitis and chorionic plate vasculitis (figure 3). Acute intervillositis and peripheral funisitis may be present, either due to inframicrobial agents or fungi, like Listeria or Candida[12].

[5] Alan T N Tita and William W Andrews, "Diagnosis and Management of Clinical Chorioamnionitis," *Clinics in Perinatology* 37, no. 2 (June 2010): 339–54, doi:10.1016/j.clp.2010.02.003.

[6] J. W. Riggs and J. D. Blanco, "Pathophysiology, Diagnosis, and Management of Intraamniotic Infection.," *Seminars in Perinatology* 22, no. 4 (August 1998): 251–59.

[7] O. Apantaku and V. Mulik, "Maternal Intra-Partum Fever.," *Journal of Obstetrics and Gynaecology : The Journal of the Institute of Obstetrics and Gynaecology* 27, no. 1 (January 2007): 12–15, doi:10.1080/01443610601016644.

[8] W M Curtin et al., "Accuracy of Signs of Clinical Chorioamnionitis in the Term Parturient," *J Perinatol* 33, no. 6 (June 2013): 422–28.

[9] Rafli van de Laar et al., "Accuracy of C-Reactive Protein Determination in Predicting Chorioamnionitis and Neonatal Infection in Pregnant Women with Premature Rupture of Membranes: A Systematic Review," *European Journal of Obstetrics & Gynecology and Reproductive Biology* 147, no. 2 (December 2009): 124–29, doi:10.1016/j.ejogrb.2009.09.017.

[10] Vincenzo Zanardo et al., "Relationship between the Neonatal White Blood Cell Count and Histologic Chorioamnionitis in Preterm Newborns," *The Journal of Maternal-Fetal & Neonatal Medicine* 25, no. 12 (December 1, 2012): 2769–72, doi:10.3109/14767058.2012.712562.

[11] Claudia Holzman et al., "Histologic Chorioamnionitis and Preterm Delivery.," *American Journal of Epidemiology* 166, no. 7 (October 1, 2007): 786–94, doi:10.1093/aje/kwm168.

[12] http://www.pathologyoutlines.com/topic/placentachorioamnionitis.html, 16 December, 2014

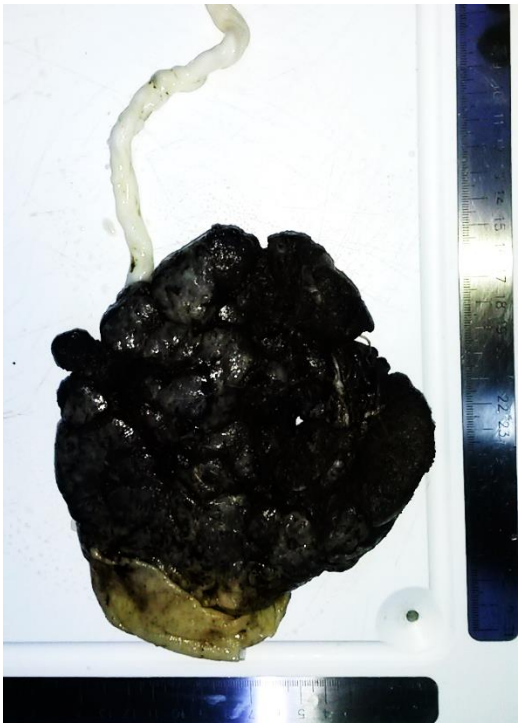

**Figure 1. - Acute chorioamniotitis placenta from a 33 weeks gestation with parenchima necrosis, purulent exsudate over amniotic membranes and haemorrhage (personal collection).**

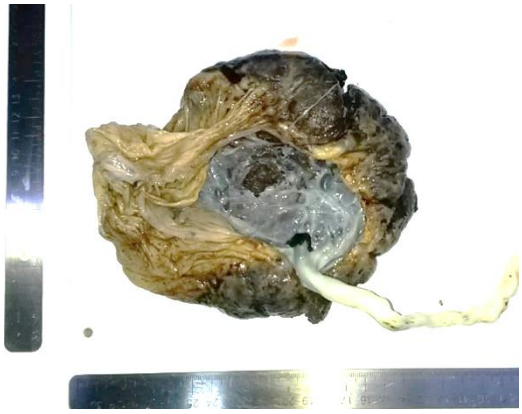

**Figure 2. Chorioamnionitis in a placenta from a 36 weeks gestation. Membranes with a yellow discoloration, necrosis and diffuse haemorrhage (personal collection).**

## SUBJECTS AND METHODS

This study purpose is to identify and to investigate the simple variability of pathologist observers participating in evaluating the degree of lesions in 10 female patients with inflammatory placentas, admitted in the Department of Obstetrics and Gynecology of University Emergency Hospital Bucharest, between 1st January 2012 and 1st February 2013, in 50 slides of archived paraffin tissue blocks, routine stained in haematoxylin-eosin, sectioned in 3 µm thickness. High resolution photographs were taken from the slides, in those areas with most obvious pathologic modifications of the placenta, with all microscopic objectives (4x10, 40x10, and 10x10) and used for data collection. The factors quantified for interobserver statistics were: acute inflammation degree, the extent of hyaline depositions, extent of fibrosis, necrosis and hyperemia. We used the Likert scale, frequently used in

pathology kappa statistics, with a classical five factor type, depicted in Table 1, following the experimental model used by other pathologists in different studies[13]. Calculations have been made using Excel spreadsheet with the aid of Cronbach's alpha and kappa statistics, superior to Kuder and Richardson formula, which can be used with non-dichotomous, continuous data.

| Factor used in study | Surface percentage: | Reliability (alpha statistics) |
|---|---|---|
| 1 = inexistent / very low frequency (widespread) of lesion | <10% | 0.92 |
| 2 = low frequency / widespread of lesion | 10-30% | 0.31 |
| 3 = moderate frequency / widespread of lesion | 30-50% | 0.81 |
| 4 = moderate-severe frequency / widespread of lesion | 50-70% | 0.46 |
| 5 = severe frequency / widespread of lesion | 70-100% | 0.61 |

**Table 1. Likert scale showing factors used in our study for evaluation of pathologic findings.**

## RESULTS

The patients were diagnosed with 6 chronic CHO (mean gestational age of 26.9 weeks) and 4 acute CHO cases (mean gestational age of 19 weeks). The placentas were sampled from women with a mean age of 29 years, in a range from 15 to 43 years old, and a mean gestational age of 23.7 weeks old, within a range from 6 to 36 weeks. In these cases, one woman was admitted with CHO with an abscess formation and one case with obvious amniotic membrane involvement in inflammatory process, with purulent exudate and lacerations.

In the ten cases that were selected, we observed 4 abortions (mean gestational age of 13.5 weeks), 5 premature newborn babies (mean gestational age of 29.2 weeks) and 1 term, aged 37 weeks (figure 3, figure 4).

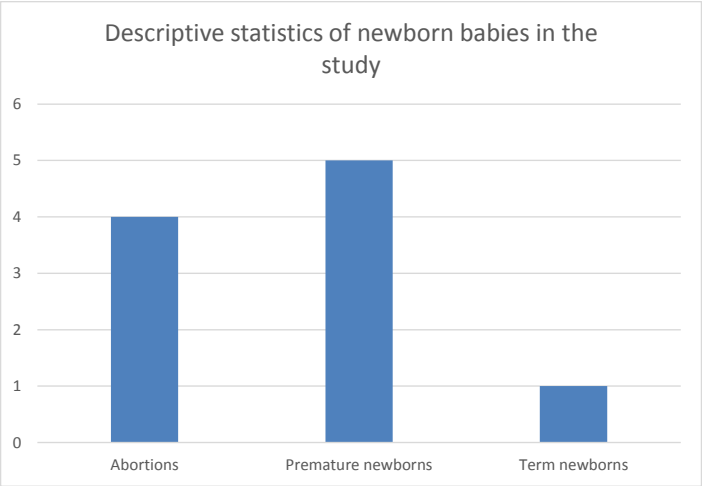


**Figure 3. Newborn statistics in studied group.**

[13] Martin Simmonds et al., "Intraobserver and Interobserver Variability for the Histologic Diagnosis of Chorioamnionitis," *American Journal of Obstetrics and Gynecology* 190, no. 1 (January 2004): 152–55, doi:10.1016/S0002-9378(03)00870-6.

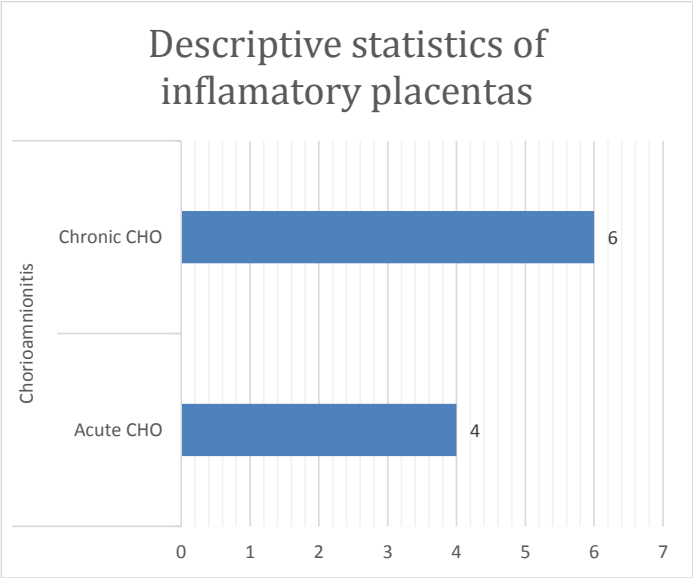


**Figure 4. Chorioamniotitis dichotomy in studied group.**

The appreciation of these lesions by the observers implied in this trial proved no agreement in between, with lowest kappa, (0.02) for acute inflammation detection, and highest kappa for hyaline (0.20) as this lesion becomes far obvious at the gestational ages for our cases (Table 2). The p-values, with one exception in acute inflammation ($p<0.66$), indicates a strong presumption against null hypothesis, showing that obtaining the detected kappa values by random chance is very unlikely ($p<0.05$). The reliability tests proved acceptable in appreciating almost inexistent or moderate extent of lesions for all factors of study, as depicted in Table 1, while low frequency lesions assessment was considered unsatisfactory.

| Statistics | Acute inflammation | Hyaline | Fibrosis | Necrosis | Hyperemia |
|---|---|---|---|---|---|
| kappa | 0.021 | 0.208 | 0.147 | 0.105 | 0.142 |
| p-value | 0.665 | 0.0003 | 0.006 | 0.025 | 0.004 |

**Table 2 Kappa statistics for agreement between observers in lesions quantifing.**

## DISCUSSIONS

The overall tendency was either to underestimate the existence of lesions, either to overestimate them, while moderate lesions were, paradoxically easy to recognize and asses equally for all factors (figure 5), probably due to their high incidence among amniotic inflammation. The unreliability of agreement of implied observers is mainly affected by many subjective, ubiquitous factors, like little experience in detecting vasodilation anomalies, interpreted as active hyperemia (figure 6), hyaline deposition (figure 7), or stromal hypercellularity for some tissue samples, while a slight involvement increase for these elements, mentioned above, could become pathologically important.

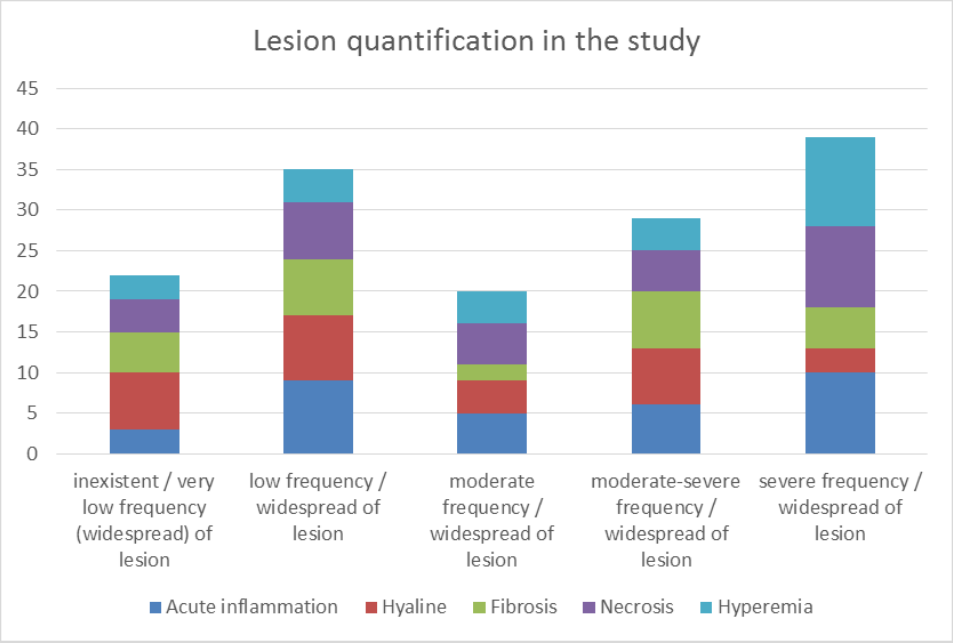


**Figure 5. - Descriptive statistics of lesion quantification using Likert scales for proposed factors.**

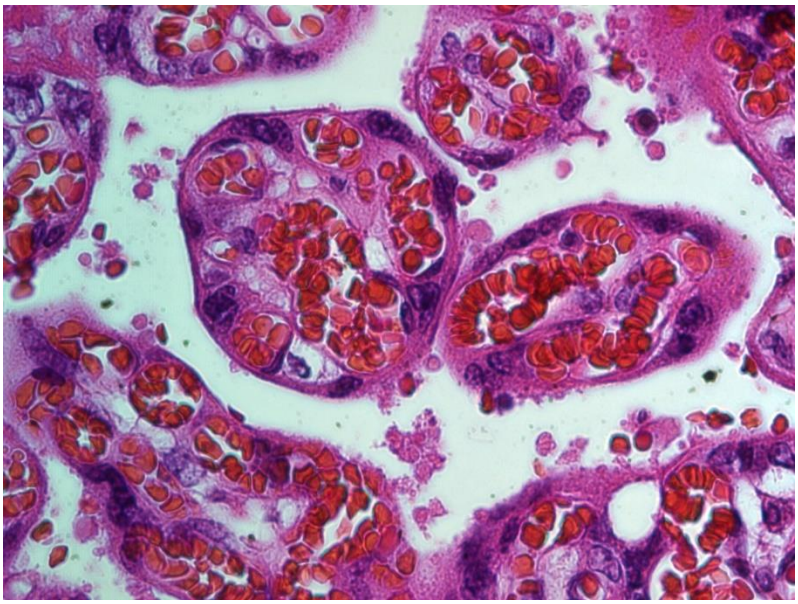

**Figure 6. - Villi in a term gestation placenta (37 weeks), with vasculosyncitial knots, interpreted as hyperemia due to hypoxic mechanism (personal collection).**

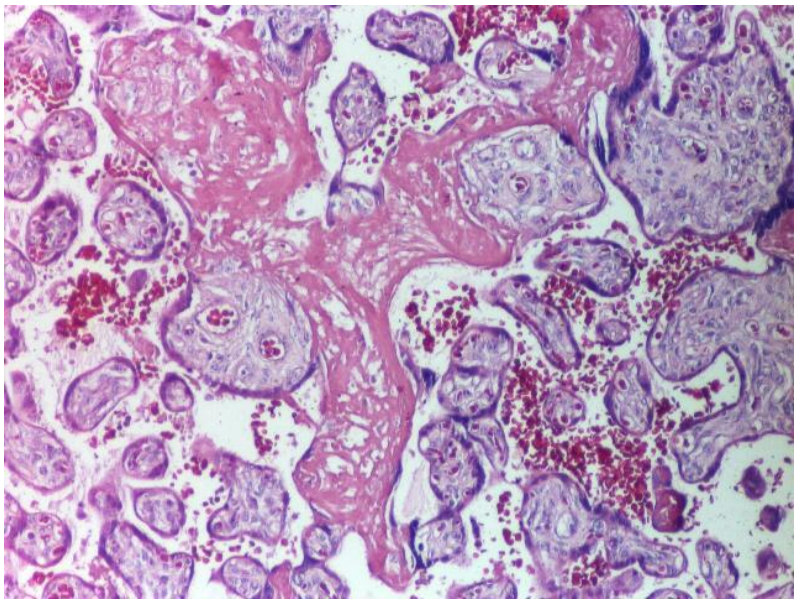

**Figure 7. Fibrnioid and hyaline deposition inside the intervillous space, in a second trimester (22 weeks) normal placenta (personal collection).**

Nowadays, significant discordance of diagnosis for placenta histologic abnormalities, like hydatidiform mole, observed by different authors, tend to persist as new criteria are required for more accurate classification of diseases[14]. In some situations, the degree of acute inflammation is difficult to evaluate as many sampling and surgical artifacts may afflict the lesions reality in the tissue, while stromal cells may undergo different morphological modifications that might become, at some point, physiological. As seen in figure 5, hyaline and fibrosis are ubiquitous in all examined samples, while necrosis volume correlates with the amplitude of inflammation.

Tissue necrosis and fibrinoid, present outside of an inflammatory process, may be unspecific and normal, as the gestation approaches term[15]. The use of immunochemistry in identifying leucocytes increases significantly diagnosis accuracy, especially of CD3+, CD4+ and CD8+ positive cells in a moderate amount, and almost no CD20+ and CD56+ positive cells, for chronic CHO[16], while Hoffbauer cells expression, positive for CD68 monocyte and macrophage markers, decreases significantly[17].

## CONCLUSION

As the difficulty of placental histopathology diagnosis increases, the necessity of implementing a reliable microscopic grading protocol arises. In conclusion, at the present moment, although routine HE sections examination remains reliable, observer variability

[14] Masaharu Fukunaga et al., "Interobserver and Intraobserver Variability in the Diagnosis of Hydatidiform Mole.," *The American Journal of Surgical Pathology* 29, no. 7 (July 2005): 942–47.

[15] Baergen, N. Rebecca et al., "Manual of Pathology of the Human Placenta", Second Ed, Springer, New York, 2011, doi: 10.1007/978-1-4419-7494-5.

[16] S. M. Jacques and F. Qureshi, "Chronic Chorioamnionitis: A Clinicopathologic and Immunohistochemical Study.," *Human Pathology* 29, no. 12 (December 1998): 1457–61.

[17] Marie-Therese N. Vinnars et al., "The Number of CD68(+) (Hofbauer) Cells Is Decreased in Placentas with Chorioamnionitis and with Advancing Gestational Age.," *Pediatric and Developmental Pathology : The Official Journal of the Society for Pediatric Pathology and the Paediatric Pathology Society* 13, no. 4 (August 2010): 68, doi:10.2350/09-03-0632-OA.1.

remains high in assessing frequent lesions and inflammation amplitude. This could be corrected with the aid of immunohistochemical identification of polymorphonuclears in tissue and the use of semi-automatic, computer assisted, histologic scanning and measurements, due to artificial intelligence algorithms.

**AKNOWLEDGEMENTS**

Special thanks go to the funded Doctoral School from the University of Pharmacy and Medicine „Carol Davila" and to the Bucharest University Emergency Hospital, Department of Gynecology and Obstetrics and the Department of Pathology. Our special thanks go, also, to the medical students at UMF „Carol Davila" , The Faculty of General Medicine, in third year, Ioana Sonia Iordache, Ana Maria Căpățînă, Larisa Catrinescu, Magda Iorgulescu, Antonia Sima, Victor George Chiper and Bogdan Gonț, for volutary aid in accomplishing data collecting and calculations for this study.