

# Role of Chemical Biomarkers in Prediction of Intra-Amniotic Infection in Cases with Preterm Premature Rupture of Membrane, Observational Study

Ibrahim Saif El-Nasr

Obstetrics and Gynecology Departments

**\*Corresponding author**

Ibrahim Saif El-Nasr MD, Department of Obstetrics and Gynecology, Faculty of Medicine, Menoufia University, Shebin el kom, Menoufia, Egypt; Tel: 01003086747; E-mail: Ibrahimalisaifelnasr@gmail.com

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**Abstract**

**Introduction:** Early recognition of subclinical intra-amniotic infection before development of clinical Chorioamnionitis dramatically improve neonatal outcome before affection of fetal neurological function.

**Objective:** this study was conducted to evaluate the role of procalcitonin and interleukin-6 in early prediction of intrauterine infection in pregnant women with premature rupture of membranes.

**Methods:** This observational prospective cohort study was conducted on 100 pregnant women with preterm premature rupture of membrane (PPROM), Patients were divided into 2 groups: **Group I:** with normal CRP and WBCs level. **Group 2:** with subclinical infection which was detected by elevated WBCs count  $>15,000$  c/mm<sup>3</sup> and / or positive CRP. This group was divided into two groups (**Group (II)** and **Group (III)**) according to development of Chorioamnionitis. follow up of these patients was done to detect the cutoff value of procalcitonin and interleukin-6 as a predictive indicator of clinical intra-amniotic infection in patients with premature rupture of membrane.

**Results:** This study showed that the mean value of maternal serum PCT concentration was higher in patients with clinical infection than its concentration in patients without infection or with subclinical infection with P-value 0.0001 which is highly significant between the studied cases and with cutoff value was  $>0.67$ ng/ml, sensitivity, specificity, PPV, NPV was 88.7%, 42.9%, 79.7% and 60% respectively and the mean value of maternal serum IL-6 concentration was also higher in patients with clinical infection than its concentration in patients without infection or with subclinical infection with P-value 0.001 which is highly significant between the studied cases and with cutoff value was  $>11.1$ pg/ml, sensitivity, specificity, PPV, NPV was 67.9%, 61.9%, 81.8% and 43.3% respectively.

**Conclusion:** maternal serum procalcitonin is a good predictor of clinical intra-amniotic infection with good sensitivity, specificity, PPV, NPV which is nearly good as CRP which is better than serum interleukin-6 regarding sensitivity and NPV.

**Keywords:** Preterm premature rupture membranes, Procalcitonin, Interleukin-6, C-reactive protein and Chorioamnionitis

**Abbreviations**

**CRP** : C-reactive protein.  
**CTG** : Cardiotochography.  
**P ProCT:** Procalcitonin.  
**RDS** : Respiratory distress syndrome.  
**Cs** : Caesarian Section  
**ROM** : premature rupture of membrane.  
**WBCs** : White blood cells.  
**Il-6** : Interleukin-6.

**Introduction**

Premature rupture of membranes (PROM) is common obstetric complication caused by multifactorial mechanisms [1]. There is great evidence considering lower genital tract infection with resultant cytokine activation is the main cause [2]. It is directly related to neonatal morbidity and about 20% of perinatal mortality [3].

One of the most common complications of PROM is Chorioamnionitis [4]. Subclinical Chorioamnionitis is an infection of the products of conception without clinical signs or symptoms of disease [5].

Clinical Chorioamnionitis is diagnosed when there is fever ( $37.8^{\circ}\text{C}$ ) plus any two of the following: Maternal tachycardia, fetal tachycardia, and uterine tenderness, foul odor of amniotic fluid or

maternal leukocytosis Account for 4.2-10.5% [6,7].

Procalcitonin (PCT) is a 116-amino-acid peptide precursor of calcitonin [8]. Which released from all tissue cells under the influence of Microbial infections and can be detected in the plasma 2 h after endotoxins, rising within 6–8 h and reaching a plateau within 20–72 h [9]. Interleukin-6 is a polypeptide composed of 183- 185 amino acids and produced by T and B cells and tightly related to inflammatory process [10].

Serial WBC & CRP commonly used in clinical practice and the only laboratory method which considered the gold standard for diagnosis of Chorioamnionitis is amniotic fluid culture which is invasive so this study was conducted to evaluate the role of procalcitonin and interleukin-6 in early prediction of intrauterine infection in pregnant women with premature rupture of membranes.

### Material and method

This observational prospective cohort study was conducted in the Obstetrics and Gynecology department at Menoufia university hospital, Shibin El-kom city, Menoufia governorate during the period between February 2017 and January 2018.

The study protocol was reviewed and approved by the local ethics committee at Menoufia University hospital and informed consent was obtained from all participants prior to commencing the study.

### Research Question

In patient with premature rupture of membrane are the procalcitonin and interleukin-6 can be used to predict intra-amniotic infection as reliable as c reactive protein?

In our study we excluded Women with multiple pregnancies, Clinical signs of infection as (rise of the temperature above 38 in two successive measurements, abdominal tenderness), Signs of active labor as (regular uterine contractions, cervical dilatation > 3 cm, cervical effacement), Maternal disease such as diabetes, urinary tract infection and fetal complications such as intra-uterine fetal death, intra-uterine growth restriction, congenital fetal malformation.

We included 100 pregnant women with PPRM selected from inpatient wards with Singleton pregnancies and confirmed Rupture of membranes.

all patients were subjected to complete History taking, General examination, Abdominal examination, obstetric examination and Obstetric ultrasound followed by Pelvic examination in the examination room under complete aseptic conditions, while the patient was put in lithotomy position, vaginal examination was done using a sterile Cusco's speculum, visualizing the cervix and vagina to confirm the leakage of the amniotic fluid, the color of the fluid and to assess the cervical dilatation and effacement.

### Patients were divided into 2 groups:

**Group I:** with normal CRP and WBCs level.

**Group2:** with subclinical infection which was detected by elevated WBCs count >15,000 c/mm<sup>3</sup> and / or positive CRP. This group was divided into two groups (**Group (II)** and **Group (III)**) according to development of Chorioamnionitis.

Blood samples were obtained from them before antibiotic administration to detect the WBCs, CRP, procalcitonin and interleukin-6 levels and then follow up of these patients was done to detect the cutoff value of procalcitonin and interleukin-6 as a predictive indicator of clinical intra-amniotic infection in patients with premature rupture of membrane.

Dexamethasone was used for lung maturation in suspected preterm babies 6mg/12 hour for 4 doses. Erythromycin capsule 250 mg every 6 h was used as local protocol.

### Statistical Analysis

- The data collected were tabulated & analyzed by SPSS (statistical package for the social science). Chi- square test was used to measure association between qualitative variables. Student t-test was used to compare mean and SD (standard deviation) of 2 sets of quantitative normally distributed data, while Mann Whitney test was used when this data is not normally distributed. Pearson's correlation was used to study correlation between two variables having normally distributed data, while Spearman's correlation was used when this data is not normally distributed. The ROC (Receiver Operating Characteristic) curve was done to detect the cutoff value with highest sensitivity and specificity, +ve and -ve predictive values were calculated. P- Value.

### Results

Our prospective study was conducted on 100 pregnant patients with premature rupture of membranes (PROM), 26 healthy patients (group I) and 74 with subclinical infection; 21 without Chorioamnionitis (group II) and 53 developed Chorioamnionitis (group III).

Patients were in the age group between 19-41 years (Mean 28.22 and  $\pm$ SD 5.37). 25 women were primigravida ( 25%) and 75 women were multigravida (75%). 30 women had previous cesarean section (30%) and 70 women had no Cs (70%). 13 women had previous PROM( 13%), 23 women had previous abortion (23%) , 24 women had negative CRP ( 24% ) and 56 women had positive CRP (56%). 19 women had normal WBCs count (19%) and 81 women had high CRP (81%).

In our study the value of WBCs ranged from 4400.0 to 39100.0 c/ mm<sup>3</sup> with the mean value of WBCs 17871.0 c/mm<sup>3</sup> ( $\pm$ SD7260.94). The value of procalcitonin ranged from 0.17 to 8.77 ng/ ml with the mean value of 1.38 ng/ ml ( $\pm$ SD 1.8). The value of interleukin-6 ranged from 0.32 to 15 pg/ ml with the mean value of 9.66 pg/ ml ( $\pm$ SD 4.17). the value of CRP ranged from 0 .2 to 98 mg/dl with the mean value of 25.54 mg/dl ( $\pm$ SD 32.5) .and the duration of PROM ranged from 2 to 216 hours with the mean value of 60.89 hours( $\pm$ SD 48).

**Table 1: Shows high statistically significant difference between Group(II) and Group(III) as regarding temperature, WBCs, CRP, procalcitonin, IL-6 and duration of PPROM and no statistically significant difference as regard of age as shown in Table 1.**

	Patient Groups				T	P value	Significant
	Group(II) with subclinical infection without chorioamnionitis (n=21)		Group(III) with clinical infection with chorioamnionitis (n=53)				
	Mean	±SD	Mean	±SD			
Age (years)	27.57	5.15	28.26	5.38	0.469	0.639	NS
WBCs (c/mm <sup>3</sup> )	17419.05	900.9	22249.06	6209.2	3.829	0.0001	HS
Temperature	37.48	0.51	37.89	0.38	3.526	0.0001	HS
CRP (mg/dl)	10.65	2.01	2.01	43.5	5.522	0.0001	HS
Procalcitonin (ng/ml)	0.69	0.22	2.14	2.34	3.857	0.0001	HS
IL-6 (pg/ml)	7.96	4.57	11.59	3.18	3.308	0.001	HS
duration of PPROM (hours)	46.14	26.11	3.8	2.0	3.591	0.0001	HS

NS=Non Significant HS=Highly Significant

There was significant correlation detected between procalcitonin versus temperature and IL-6 and highly significant correlation with duration of PROM, CRP and WBCs by using Pearson's correlation test as shown in table 2.

**Table 2: Correlation study between Procalcitonin and interleukin-6 with different numerical parameters of studied patients**

Variable	Procalcitonin (ng/ml)			IL6(pg/ml)		
	R	P value	Sig.	R	P value	Sig.
Temperature(°C)	0.386	0.000	S	0.418	0.000	S
duration of PPROM(hours)	0.689	0.000	HS	0.319	0.001	S
IL6(pg/ml)	0.269	0.007	S	0.269	0.007	S
CRP(mg/dl)	0.689	0.000	HS	0.269	0.007	S
WBCs (c/mm <sup>3</sup> )	0.552	0.000	HS	0.287	0.004	S

S=Significant HS=Highly Significant

There was significant correlation detected between interleukin-6 versus different variables by using Pearson's correlation test as shown in table 3. High statistically significant difference as regard CRP of neonates, no statistically significant difference as regard, gestational age at PROM, NICU

**Table 3: Neonatal outcome**

			Patient groups		P value	Significant
			Group(II) (n=21)	Group(III) (n=53)		
CRP of neonates	Negative	N	2	11	0.001	HS
		%	9.5%	20.8%		
	Positive	N	19	42		
		%	90.5%	79.2%		
gestational age at PROM	Pre-term	N	12	33	0.684	NS
		%	57.1%	62.3%		
	Full-term	N	9	20		
		%	42.9%	37.7%		
NICU	Negative	N	1	17	0.893	NS
		%	4.8%	32.1%		
	Positive	N	20	36		
		%	95.2%	67.9%		
		%	23.8%	26.4%		

Table (4) Shows no significant difference according to procalcitonin among patient of group II (**P-value** 0.979). Significant difference according to procalcitonin among patient of group III (**P-value** 0.012) no significant difference according to interleukin-6 among patient of group II (**P-value** 0.972) no significant difference according to interleukin-6 among patient of group III (**P-value** 1.000).

**Table 4**

	Group II		t	Post hoc P-value	Significant
	Pre-term	Full-term			
Procalcitonin (ng/ml)	0.66±0.24	0.73±0.19	20.4	0.979	NS
IL-6 (pg/ml)	7.3±4.25	9.02±5.18	12.97	0.972	NS
	Group III		t	Post hoc P-value	Significant
	Pre-term	Full-term			
Procalcitonin (ng/ml)	0.68±0.24	1.1±0.59	20.4	0.012	S
IL-6 (pg/ml)	11.59±3.1	11.59±3.39	12.97	1.000	NS

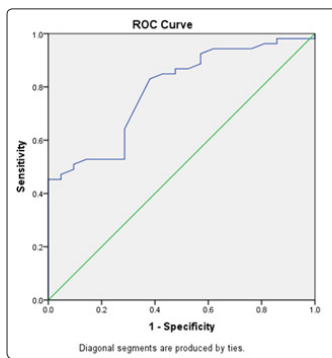
NS=Not Significant

S=Significant

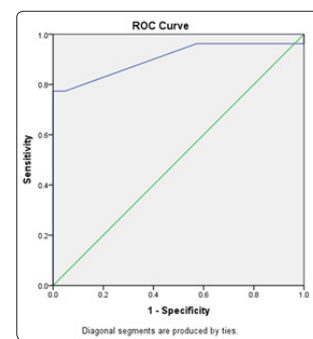
table (5) shows the area under the ROC curve is 0.904, the cutoff value of CRP >1.15 mg/dl with sensitivity 96.2% and specificity 42.9% with positive predictive value (PPV) 81% and negative predictive value(NPV) 81.8. the area under the ROC curve is 0.789, the cutoff value of procalcitonin >0.67 ng/ml with sensitivity 88.7% and specificity 42.9% with positive predictive value (PPV) 79.7% and negative predictive value(NPV) 60%. This table shows the area under the ROC curve is 0.748, the cutoff value of interleukin-6 >11.1 pg/ml with sensitivity 67.9% and specificity 61.9% with positive predictive value (PPV) 81.8% and negative predictive value(NPV) 43.3%.

**Table 5: ROC curve analysis for procalcitonin and interleukin-6 in prediction of clinical intra-amniotic infection**

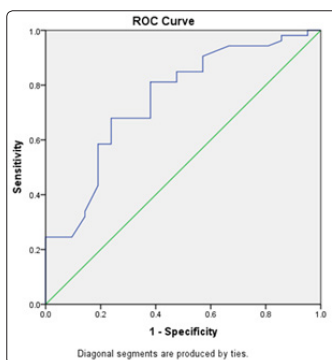
	Area Under the Curve						
	AUC	P value	Best cut off point	Sensitivity	Specificity	PPV	NPV
Procalcitonin	0.789	0.000	0.67	88.7	42.9	79.7	60
interleukin-6	0.748	0.001	11.1	67.9	61.9	81.8	43.3
CRP	0.904	0.000	1.15	96.2	42.9	81	81.8



**Figure 1:** ROC curve shows the specificity and sensitivity of ProCT in detection of clinical intra-amniotic infection



**Figure 3:** ROC curve shows the specificity and sensitivity of CRP in detection of clinical intra-amniotic infection



**Figure 2:** ROC curve shows the specificity and sensitivity of interleukin-6 in detection of clinical intra-amniotic infection

### Discussion

Obstetricians all over the world don't have agreement about the protocol for management of PROM but antibiotic prophylaxis and steroid therapy remain the standard management for PROM before 34 weeks of gestation [11].

Early recognition of subclinical intra-amniotic infection before development of clinical Chorioamnionitis dramatically improve neonatal outcome before affection of fetal neurological function [12].

for diagnosis of Chorioamnionitis multiple Inflammatory cytokines can be measured in amniotic fluid through amniocentesis but this procedure have complications like intrauterine infection decreased amniotic fluid volume and cannot be repeated regularly Therefore,

easy, safe and repeatable methods are required in clinical practice [13].

Repeated measurements of WBC & CRP are traditionally used for prediction of subclinical intra-amniotic infection. Recently PCT and interleukin-6 have been investigated as markers for early prediction of subclinical Chorioamnionitis [14].

This current study was conducted on 100 pregnant women with PROM where Blood samples were obtained from them before antibiotic administration to detect the CRP procalcitonin and interleukin-6 levels and then follow up of these patients to detect the cutoff value of procalcitonin and interleukin-6 as a predictive indicator of clinical intra-amniotic infection in patients with premature rupture of membrane.

This study showed that the mean value of maternal serum PCT concentration was higher in patients with clinical infection than its concentration in patients without infection or with subclinical infection with P-value 0.0001 which is highly significant between the studied cases and with cutoff value was  $>0.67\text{ng/ml}$ , sensitivity, specificity, PPV, NPV was 88.7%, 42.9%, 79.7% and 60% respectively and the mean value of maternal serum IL-6 concentration was also higher in patients with clinical infection than its concentration in patients without infection or with subclinical infection with P-value 0.001 which is highly significant between the studied cases and with cutoff value was  $>11.1\text{pg/ml}$ , sensitivity, specificity, PPV, NPV was 67.9%, 61.9%, 81.8% and 43.3% respectively.

As regarding procalcitonin our results consistent with the results reported by Oludag et al which include 120 patients (32) PPRM, (35) PROM at term (24) healthy women at preterm gestation and (30) healthy women at term who found that sensitivity and specificity were 92.3% and 68.4% respectively and with PPV was 66.7% and NPV was 92.9% with cutoff value  $0.54\text{ng/ml}$  and P-value of the CRP level of the patients was 0.05 [15].

The study result also consistent with the results reported by Ivana Maurac et al which include 120 mothers with singleton pregnancies were enrolled in their study. 60 pregnancies were complicated with a premature rupture of membranes, and 60 were control pregnancies. As regards CRP the best cut-off  $>7\text{mg/L}$ , 82.8% sensitivity, 71.4% specificity and PCT the best cut-off  $0.053\text{ng/L}$ , 69% sensitivity, 73.6% specificity [16].

Also the results consistent with the results reported by Sharareh Seivani et al which include 48 pregnant women with PROM and their neonates. A significant correlation was observed between women who had histopathology confirmed Chorioamnionitis and neonate hospitalization in the neonatal intensive care unit ( $P<0.001$ ). The sensitivity, specificity, positive and negative predictive values of PCT inflammatory indices were 100%, 79%, 57.5%, and 100%, respectively [17].

The results of our study are different from that reported by Trobe which include 142 patients 48 patients with PPRM, 30 patients with PROM at term, 31 healthy women at preterm gestation and 33 healthy women at term which was the value of maternal plasma procalcitonin determinations in the diagnostics of PPRM cases suspected of intra-amniotic infection is unsatisfactory as its cut off value was  $.019\text{ng/ml}$  and its sensitivity was 53% and specificity

was 45% with PPV 35% and NPV 64% [18]. This difference in results may be due to small number of PPRM cases and large number of full term and healthy (without PROM) cases in relation to sample size.

As regarding IL-6 our results similar to the results reported by Pfeiffer et al which include 71 patients with PROM with a cutoff of  $11\text{pg/ml}$ , IL-6 reaches a sensitivity of 81% and a specificity of 76% positive predictive value 50% and negative predictive value 93% [19].

Our study results consistent with results reported by Shilpa Gulati et al where serial serum IL-6 levels were measured in 45 women with preterm PROM at gestation 24–34 weeks. The best cut-off value of IL-6 is  $8\text{pg/ml}$  with sensitivity 82.6% and specificity 86.3% [20].

## Conclusions

Procalcitonin and interleukin-6 are cheaper, faster and reliable sensitive markers for detection of intra-amniotic infection in premature rupture of membranes, with a high sensitivity and negative predictive value. So, they considered reasonable screening tests for detection of intrauterine infection. Also more studies are needed to compare between serum procalcitonin and other markers like prolidase and total oxidative status (TOS).

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## References

1. Sagol S, Sagol O, Ozkal S, Asena U (2001) Increased apoptosis in premature rupture of the fetal membranes. *Neonatal Med* 2: 9-12.
2. Andrews W, Hauth J, Goldenberg R (2000) Infection and preterm birth. *Am J Perinatol* 17: 357-365?
3. Nava Flores J, Enriquez M, Hernandez-Valencia M (2003) Maternal and fetal morbidity in patients with premature rupture of the membrane after 37-week gestation, Causes and Costs. *Gynecol Obstet Mex* 71: 343-348.
4. Elarth M, Elizabeth N, Allred M, Alon levition (2003) Prolonged latency after preterm premature rupture of membranes. *Am J Obstet Gynecol* 189: 794-798?
5. Arias F (2008) Preterm parturition syndrome, preterm labor: Practical guide to high-risk pregnancy and delivery rd. Elsevier India 181-84; 200-202.
6. Cunningham F, Kenneth J, Steven L, John C, Hauth, et al. (2005) Williams Obstetrics; 22nd edition, Infections. F. Gary Cunningham 1275-1326.
7. Osmanagaoglu M, Unal S, Bozkaya H (2005) Chorioamnionitis risk and neonatal outcome in preterm premature rupture of membranes. *Arch Gynecol Obstet* 271: 33-339.
8. Van Rossum A, Wulkan R, Quedusluys Murphy A (2004) Procalcitonin as an early marker of infection in neonates and children. *Lancet Infect Dis* 4: 620-630.
9. Muller B, White J, Nylen ES, Snider R, Becker K, et al (2001) Ubiquitous expression of the calcitonin-i gene in multiple tissues in response to sepsis. *J Clin Endocrinol Metab* 86: 396-404.
10. Oleszczuk J, Wawrzycka B, Maj G (1997) Interleukin-6 and neopterin levels in serum of patients with preterm labor with and without infection *European J obstet Gynecol and Reproductive*

Biology 74: 27-30.

11. Tita A, Andrews W (2010) Diagnosis and management of clinical chorioamnionitis. Clin Perinatol 37: 339-354.
12. Wu Y, Colford JM Jr (2000) Chorioamnionitis as a risk factor for cerebral palsy. A meta-analysis. Journal of American Medical Association 284: 1417-1424.
13. Cherouny P, Pankuch G, Botti J, Appelbaum P (1992) The presence of amniotic fluid leukoattractants accurately identifies histologic chorioamnionitis and predicts tocolytic efficacy in patients with idiopathic preterm labor. AM J Obstet Gynecol 167: 683-688.
14. Di Naro E, Ghezzi F, Raio L, Michel D, Francesesco, et al. (2003) reactive protein in vaginal fluid of patient with preterm premature rupture of membranes. Acta Obstet Gynecol 82: 1072 -1079.
15. Tülay Oludag, Funda Gode, Erkan Caglayan, Bahadır Saatli, Recep Emre Okyay, et al. (2014) Value of maternal procalcitonin levels for predicting subclinical intra-amniotic infection in preterm premature rupture of membranes, Journal of Obstetrics and Gynaecology 40: 954-960.
16. Snjezana Gveri C, Vesna Gasparovic, Ivana Maurac, Sonja Jurica, Petrana Beljan (2014) Procalcitonin vs C-reactive protein in early detection of intrauterine infection in premature rupture of membranes and neonatal infections. Signa Vitae 9: 54-57.
17. Sharareh Seivani, Farzaneh Broumand, Siamak Naji (2017) Predictive Value of Maternal Serum Level of Procalcitonin in Diagnosing Chorioamnionitis in Mothers with Preterm Premature Rupture of Membrane. Internal Medicine and Medical Investigation Journal 2: 149-154.
18. Andrzej Torbé (2007) Maternal Plasma Procalcitonin Concentrations in Pregnancy Complicated by Preterm Premature Rupture of Membranes. Mediators of Inflammation 2007: 35782.
19. Kerstin A Pfeiffer, Jochen Reinsberg, Anjarahmun, Jan Schmolling, Dieter Krebs (1999) Clinical application of maternal serum cytokine determination in premature rupture of membranes – interleukin-6, an early predictor of neonatal infection. Acta Obstet Gynecol Scand 78: 774-778.
20. Shilpa Gulati, Swati Agrawal, Chitra Raghunandan, Jayashree Bhattacharya, Arvind Saili, et al. (2012) Maternal serum interleukin-6 and its association with clinicopathological infectious morbidity in preterm premature rupture of membranes. The Journal of Maternal-Fetal & Neonatal Medicine 25: 1428-1432.

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