Umbilical cord blood procalcitonin level in early neonatal infections: a 4-year university hospital cohort study

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Umbilical Cord Blood Procalcitonin Level in Early Neonatal Infections: A 4-year University Hospital Cohort Study.

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Abstract: **Purpose**. Study of Procalcitonin (PCT) measured in cord blood as a discriminating marker of early-onset neonatal infection. **Methods**. Monocenter retrospective study with prospective collection of data including all babies born during the study period. Those presenting infection risk factors had PCT measurement. Three groups were defined : certainly infected, probably infected, and non-infected. **Results**. 12485 newborns were included, 2151 had PCT measurement, 26 were infected. Receiver operating curves of PCT determined 0.6 ng/ml as the best cut-off, with an area under the curve of 0.96 (CI 95%: 0.95 - 0.98). Sensitivity, specificity, positive and negative predictive value and positive and negative likelihood ratios were 0.92 (0.75 - 0.98), 0.97 (0.96 - 0.98), 0.28 (0.20 - 0.36), 0.99 (0.99 - 0.99), 32 (24 - 41) and 0.08 (0.02 -0.3). Post-test probabilities were 28% (23 to 33) if the test was positive, and less than 0.01% (0 - 1.10-5) if the test was negative. Gestational age between 28 and 32 weeks (OR 4.4, 1.2 - 16.2) and pH at birth < 7.10 (OR 2.9, 1.1 - 7.4) were other independent factor of PCT increasing ($p<0.05$). **Conclusion**. PCT measured in umbilical cord blood is reliable to detect early infected and non-infected newborns.

Keywords: Early onset neonatal infection; neonate; procalcitonin

INTRODUCTION

Early-onset neonatal infection (EONI) suspicion is one of the most stressful situations for neonatologists, despite generalized prophylaxis of group B streptococcal (GBS) neonatal infection (1-3). Indeed, despite the reduced frequency of this pathology, the diagnosis remains an emergency because early diagnosis and treatment are critical to optimise the outcome (4). Because clinical

signs of EONI are known to be non specific, pediatricians often start early empiric antibiotic treatment in newborns presenting few symptoms or some risk factors of infection before receiving results of bacteriologic cultures and inflammatory markers, but as a result, exposing a considerable number of patients to unnecessary treatment. In a recent study, Stocker *et al* reported that an empiric antibiotic treatment (ampicillin and gentamicin) $\geq$ 72h was administered to 82% of a cohort of neonates with suspected EONI, while only 18% of them were finally classified as probable infections and 1% as proven infections (5). Concerns about subsequent consequences of such treatment strategies are serious and multiple: changes in microbiotal ecology of the neonates and the recently reported role of these changes in the early origins of diseases (6,7); risk of development of resistant bacteria (8); nosocomial infection risk; mother/newborn separation; and increased cost.

Currently, a sensitive and specific marker is required in order to make a definite EONI diagnosis. While most clinical and standard biological markers are inadequate (4, 9), procalcitonin (PCT), implicated as a sensitive and specific marker of bacterial infections in various situations in adult and pediatric medicine, may be a good candidate (10, 11). Some studies have shown a physiological increase in the PCT concentration during the first three post natal days, thereby making the interpretation of this marker during this period complicated (12). In this context, we previously conducted a prospective cohort study in neonates with suspected EONI, measuring the serum PCT concentration

at birth in umbilical cord blood, which reflects antenatal (transplacental or ascendant) infectious process. PCT appeared in as a discriminating marker of EONI with 87.5% (CI 95%: 71 - 100) sensitivity, 98.7% (CI 95%: 97.7 - 99.7) specificity, 67.3 positive likelihood ratio and 0.13 negative likelihood ratio (13). However, the determination of PCT concentration used a semi-quantitative method with imprecise cut-off, and confidence intervals were large because of the low incidence of EONI and a limited number of neonates with probable or proven infection.

Consequently, the aim of this study was to evaluate the diagnostic value of a quantitative umbilical cord blood PCT concentration in a large cohort of neonates in a university hospital where PCT determination is routinely performed in newborns presenting risk factors of EONI. We hypothesized that umbilical blood cord PCT quantitative concentration is an early and discriminating marker of EONI.

PATIENTS AND METHODS

The study reported here follows STARD guidelines (14). We conducted a one center observational retrospective analysis of prospectively collected data from July 2005 to September 2008 in the University Hospital of Nantes, France. Written consent was not required. The study was approved by the local clinical research committee of the University Hospital of Nantes.

Inclusion and exclusion criteria

All children born during this period were included in the study, composing an historical cohort. In our center, only those with risk factors for EONI usually have a PCT measurement and were therefore considered in the statistical analysis. These risk factors are those proposed by international and French national consensus guidelines: clinical suspicion of chorioamnionitis (fever, painful uterine contractions), intrapartum fever $\geq 38°C$, spontaneous premature delivery at < 37 gestational weeks (GW), prolonged rupture of membrane > 12 h, maternal colonization with GBS, signs of fetal asphyxia (tachycardia, meconium stained amniotic fluid) and symptomatic newborn (respiratory distress/apnea, tachycardia/bradycardia, arterial hypotension/poor perfusion, seizure/floppy infant, irritability/lethargy, feeding intolerance) (15 - 17). We add that since 2001, GBS screening in France is generalized and antibiotics are administrated to the mother during labor in case of a positive test in accordance with national guidelines (18). Newborns presenting antenatal proinflammatory malformation (gastroschisis and omphalocele) were not

included and those whose data were incomplete were secondarily excluded from the study.

Laboratory test

Procalcitonin measurement and bacteriological samples (gastric and placental culture) were performed in every newborn presenting infection risk factors. Blood samples were obtained from umbilical blood in standard vacuum blood collection tubes (Venosafe®, TERUMO) containing lithium heparin. The tubes were carried to the biochemistry laboratory within one hour and then centrifuged at 3000 g for 10 minutes before analysis. We used a time-resolved amplified cryptate emission (TRACE®) technology assay with the KRYPTOR® automated analyzer (BRAHMS, Hennigsdorf, Germany). This assay requires 50 µl of plasma, and the reaction time is about 20 minutes. The assay has a very low detection limit (0.02 ng/ml) and a functional sensitivity of 0.06 ng/ml. Reproducibility of the test is excellent, with a coefficient of variation of 6% for a value of 0.25 ng/ml and 3% for values higher than 1.0 ng/ml. If the newborn was symptomatic during the first 48 hours of life, or in the case of a positive Gram stain of a bacteriological sample, investigations were extended after birth with blood cell count, blood culture and C-Reactive protein (CRP). The latter was performed by an immunoturbidimetric method with a COBAS® 6000 analyzer (ROCHE DIAGNOSTICS, Mannheim, Germany). CRP levels were obtained during the initial evaluation of the newborn (CRP1) and between 12 and 24 hours after (CRP2).

Data collection

We systematically recorded data in a customized database at the time of newborn discharge. Clinical data included gestational age at birth, birth weight, sex, prenatal corticosteroid exposure, time of membrane rupture, mode of delivery, antenatal antibiotics exposure, chorioamnionitis, preeclampsia, intrauterine growth retardation, death, multiple pregnancy, APGAR score at 5 minutes of life and abnormal clinical signs during the first 48h of life. Biological data included the result of the mother's GBS screening, gastric fluid and placenta colonization, blood culture and cerebrospinal fluid result, pH at birth, CRP1, CRP2, white blood cell (WBC) count, immature neutrophil rate, and platelets count.

Patient classification

According to the clinical, biological and bacterial findings, and in accordance with international criteria, three groups were *a posteriori* defined as certainly infected, probably infected, and non-infected (19, 20). Two neonatologists who were unaware of the procalcitonin value and the medical management of the patient retrospectively classified newborns according to the following criteria. In cases of discordance, a third opinion was used. The certainly infected group was defined by a positive central sample (blood culture or cerebro spinal fluid). A newborn was considered as probably infected if he presented clinical signs and at least two abnormal laboratory results among WBC count > 34 10^9/l or < 5 10^9 /l, immature neutrophil > 10%, thrombocytopenia < 100 10^9 /l, and CRP > 20

mg /l when blood culture was negative. Patients were considered non-infected when their data did not match these criteria. For the analysis of results, probably and certainly infected newborns were considered infected.

Statistical analysis

We first described baseline characteristics of all patients presenting infection risk factor. We then analyzed PCT distribution among infected (certainly or probably) and non-infected newborns and we compared PCT median in these two groups using Mann and Whitney test. We then performed a ROC curve using Medicalc software in order to determine the best cut-off for PCT measured in cord blood. Sensitivity, specificity, negative and positive predicting values and negative and positive likelihood ratios were assessed using SPSS For Windows version 15.0. Post-test probabilities were determined using a nomogram of bayes (21). The abilities of PCT and CRP to discriminate neonates with and without early neonatal infection were compared using the method of comparison of AUC published by Hanley and McNeil (22). Then, in order to assess the ability of antenatal infection risk factors and PCT to predict infection, we analyzed their statistical relationship with infection performing a univariate analysis followed by a multivariate analysis by logistic regression. All the following variables were binary and were coded as follows: (0) no, (1) yes for clinical suspicion of chorioamniotis, intrapartum fever >38°C, spontaneous premature delivery < 37 GW, prolonged rupture of membrane > 12 h, maternal colonization with GB, fetal asphyxia, and PCT value higher than the cut-off.

Then, in order to compare the accuracy of these clinical antenatal risk factors and of PCT for the screening of patients, we built two mathematical models. For the building of these two models, the whole population was randomized into 2 subgroups using an SPSS 15.0 random function. We then performed a new logistic regression to select items composing the models, among the first subgroup with a significance limit of $p>0.05$ to remove from the model, and then validated it in the second subgroup. The first model was called 'clinical model' and only included clinical risk factors. The second model was called 'bio clinical model' and was the same as the first model, but also included the PCT value. The ability of each model to discriminate between infected and non infected newborns was evaluated by their AUC. AUCs of the two models were compared in each subgroup and to PCT alone using the Hanley and McNeil method (22). Additionally, for each model we compared the AUC between the two subgroups using Delong's method to assess the reproducibility of the models (23). Lastly, in order to describe more accurately all factors increasing PCT value, we performed a logistic regression with SPSS version 15.0 software.

RESULTS

Clinical and bacteriological description of the population

Overall, 12485 newborns were analysed during the study period. Three of these were certainly infected (0.24‰). As shown in Figure 1, 2178 of these were suspected of having an infection and had a PCT measurement, among which 27 were excluded because of lacking data, 2151 newborns were therefore

considered in the statistical analysis and the following results only concern these newborns. Among these 2151 newborns presenting risk factors, 26 were considered as probably or certainly infected which represented an incidence of 1.2%. In the preterm population the incidence was 2%. Baseline characteristics of the selected patients are described in Table 1.

PCT as a marker of early neonatal infection

Distribution of PCT value among infected and non infected patients is shown in Figure 2. PCT median in infected newborns was significantly higher than among non infected patients (3.03 ng/ml vs 0.16 ng/ml, $p<0.001$). Considering the entire study population, analysis of the ROC curve (Figure 3) determined a cut-off of 0.6 ng/ml as the best compromise, with an area under the curve of 0.96 (CI 95%: 0.95 - 0.98). Performing ROC curves in full-term and preterm populations we selected the same cut-off of 0.6 ng/ml. Twenty-four of the 26 infected children had a PCT value higher than 0.6 ng/ml, and PCT was lower than 0.6 ng/ml in 2066 of the 2125 non-infected newborns. Nevertheless, among the 2097 newborns presenting a negative PCT result, 2 were probably infected. The first one was a full-term baby with a membrane rupture longer than 24 hours as the only infection risk factor, presenting respiratory distress 24 hours after birth. *Escherichia coli* was found in gastric fluid culture, PCT was 0.22 ng/ml, CRP1 measured 2 hours after birth was negative but CRP2 24 hours later was at 34 mg/l and antibiotics were introduced at the beginning of the

symptoms. The second newborn was also a full term baby whose mother had fever and a membrane rupture of 14 hours, with meconium stained amniotic fluid. The newborn was asymptomatic with a PCT of 0.17 ng/ml and a negative CRP1 at birth. CRP2 18 hours later was at 87 mg/l, antibiotic treatment was therefore started. On the basis of the cut-off of 0.6 ng/ml, statistical values of PCT established in the whole cohort and in preterm and full-term populations are expressed in Table 2. Corresponding post-test probabilities determined with the nomogram of Bayes are shown in Figure 4. These results indicate that the probability for a newborn with an infection risk factor of being infected was 28% (CI 95%: 23 - 33) if the test was positive, and less than 0.001% (CI 95%: 0 - 1.10^{-5}) if the test was negative. In the preterm group, the post-test probability of being infected was 30% (CI 95%: 23 - 37) in the case of a positive test, and 0% (CI 95%: 0 - 1) in the case of a negative test.

Comparison of PCT and CRP

ROC curves of PCT, CRP1 and CRP2 are represented in Figure 3. The area under the ROC curve of CRP1 was 0.75 (CI 95%: 0.72 - 0.78), which was significantly lower than that of PCT (p=0.002). Considering the CRP2 value, the area under the ROC curve was 0.88 (0.86 - 0.91) and did not differ from that of PCT (p= 0.184) or CRP1 (p=0.056).

Relationship between infection and antenatal risk factors and PCT

As displayed in Table 3, only chorioamnionitis was identified as a statistically significant independent antenatal risk factor of infection related with post natal

diagnosis of infection ($p< 0.05$). PCT measured in cord blood was also independently related with infection ($p<0.001$).

Analysis of 'clinical' and 'bio clinical' models

In each model, the probability of the newborn being infected was $1/(1+e^{-z})$. In the 'clinical' model, z was equal to 0.092 * Gestational age (weeks) + 2.84 * clinical suspicion of chorioamnionitis (no = 0, yes = 1) + 2.3459 * APGAR score at 5 minutes less than 7 (no = 0, yes = 1) - 2.1453. For the 'bio clinical' model, z was equal to 0.064 * Gestational age (weeks) + 2.29 * clinical suspicion of chorioamnionitis (no = 0, yes = 1) + 0.73 * APGAR score at 5 minutes less than 7 (no = 0, yes = 1) + 0.97 log[PCT(ng/ml)] - 2.07. When the two models were built by being applied to the first subgroup, the AUCs were 0.90 (CI 95%: 0.84 - 0.95) for the 'clinical' model and 0.98 (0.96 - 1) for the 'bio clinical' model, with the latter being significantly higher ($p<0.05$). The AUCs were also significantly different when the models were applied to the second subgroup for validation, being 0.70 (0.62 - 0.78) for the 'clinical' model and 0.96 (0.92 - 0.99) for the 'bio clinical' model ($p<0.001$). A comparison of the AUCs of the 'clinical' model and 'bio clinical' model between the two subpopulations did not show any significant difference ($p = 0.05$ and $p = 0.54$, respectively) and confirmed the reproducibility of the models. AUC of the 'clinical' model in the whole population was not different compared to that of PCT alone ($p = 0.2$)

Analysis of other factors influencing PCT value

All results of the analysis of other factors associated with a PCT value higher than 0.6 ng/ml are expressed in Table 4. Gestational age between 28 and 32 weeks, pH at birth lower than 7.10 and post-natal diagnosis of infection were independently related with a PCT value higher than 0.6 ng/ml ($p<0.05$).

Evaluation of the impact of the use of PCT on antibiotic administration

During the study period, 348 of the 2151 studied babies presented abnormal clinical signs during their first 48 hours of life. Among the 1803 asymptomatic newborns, 105 received a treatment with antibiotics, and only 29 of them had a PCT higher than 0.6 ng/ml, and only one asymptomatic newborn with PCT lower than 0.6 ng/ml was *a posteriori* considered infected. We can conclude that if antibiotic treatment set up had been decided only considering PCT value among these asymptomatic newborns, it could have been avoided in 76 of them (72%), for only one infected newborn missed.

DISCUSSION

This study analysed a larger cohort of newborns than in the previous study (13) and confirmed that PCT measured in umbilical cord blood is an early and discriminating marker of EONI. First, its high sensitivity shows its ability to detect early infected patients. Indeed, 24 of the 26 infected patients in our cohort had a PCT value higher than 0.6 ng/ml and only 2 of these had a negative concentration. Another interesting result is the ability of this marker to detect non-infected patients among those presenting risk factors of infection as represented in the cohort of this study. Post-test probability determined with the

nomogram of Bayes showed that a newborn with at least one of these infection risk factors and a PCT value lower than 0.6 ng/ml had a statistical risk of being infected of about 0%. This confirms the results of the preliminary study carried out in a similar population with semi-quantitative dosage, where the sensitivity and negative predicting values were 0.87 (CI 95%: 0.71 - 1) and 0.99 (0.98 - 0.99), respectively. The post-test probability was 1% for a negative test (13). This also confirms that PCT can be used in full-term newborns as in preterms, with the same cut-off. In comparison, Kordek *et al.* conducted a study of 286 newborns and found a sensitivity of 0.80, and an NPV of 0.95 for PCT also measured in cord blood with a cut-off of 1.22 ng/ml (24).

The main weakness of this marker, not identified in the preliminary study but shown in this large cohort, is its low positive predictive value. Among the 84 children with a PCT value higher than 0.6 ng/ml, only 24 were infected. A low predicting value for positive measurement was already found in the aforementioned Kordek study (24). The first explanation of these results seems to be the impact of perinatal asphyxia, as shown in the logistic regression, which identified a pH lower than 7.10 as an independent factor. These findings have seldom been described previously. In a group of 117 non-infected patients, Chiesa *et al.* (12) did not find any significant influence of antenatal asphyxia on PCT measurement. The second explanation could be the influence of antenatal inflammation. Indeed, prematurity in this study increases PCT independently of infection. A specific analysis of the perinatal context of these preterms with

increased PCT, but without diagnosed infection, shows that most had several infection risk factors with a probable foetal inflammation response syndrome. We can suggest the possibility that antibiotics received by the mother avoided neonatal infection, but not labour.

As we demonstrated in the comparison of a ‘clinical’ model with a ‘bio clinical’ model integrating PCT, this marker seems useful in clarifying the risk of the newborn being infected at birth. This demonstrates that in this situation, the clinical approach considering only antenatal risk factors is not sufficient for the evaluation of the risk of infection which is more precise when adding the result of PCT. This prompts to recommend PCT measurement in cord blood in neonates presenting risk factors but this has to be validated by prospective studies. Thus, the next step in the use of PCT in clinical practice will be to validate this marker in clinical decision rules. In the context of other pediatric infectious diseases such as meningitis (25) and febrile urinary tract infections (26), PCT seems to be a useful tool for better management by the clinician. In neonatology, Franz *et al* (27) have shown that a diagnostic algorithm based on CRP and IL-8 reduced antibiotic therapy compared with a standard strategy, without increasing the rate of missed infections. According to our results, during the study period, 72% of antibiotic treatment in asymptomatic newborns could have been avoided if the decision had been guided by clinical examination and PCT measurement, missing only one infected patient. This suggests that among these asymptomatic patients, it should be possible to construct a reliable clinical

rule with a pivotal role for PCT in order to decrease the number of unnecessary neonatal antibiotic treatments. In patients presenting clinical signs of infection, it appears, at the moment, unreasonable to introduce a treatment considering only the PCT result, but this marker should be useful to stop the treatment 48 hours after introduction, after collecting all bacteriological results. This could help limit the number of newborn hospitalisations, to promote parent-infant bonding, and diminish healthcare costs. Moreover, such an antibiotic treatment limitation could reduce the adverse effects on gut microbiota and subsequent consequences on future child and adult health.

Lastly, as PCT seems to be a reliable infection marker, we can speculate on its ability to be a prognostic marker in order to evaluate early the predicted consequences of neonatal inflammation. Indeed, the relationship between neonatal inflammation, caused among other factors by perinatal infection, and pulmonary and cerebral morbidity has been well described for about ten years (28-36).

In conclusion, our study confirms the ability of PCT measured in umbilical cord blood to detect early neonatal infection, and also to determine non-infected newborns among those presenting infection risk factors. This should allow the neonatologist to integrate this marker into clinical decision rules in order to reduce unnecessary antibiotic treatment during the first days of life.

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Legends

Figure 1 : Study flow diagram

Figure 2 : Box-and whisker plot showing PCT concentration according to the infectious status. PCT level is expressed in ng/ml. Box represent first and third quartiles, horizontal lines represent medians with the corresponding numerical value, whiskers give 10/90 percentiles. Open circle represents oulier. Dotted line represents the cut-off of 0,6 ng/ml. p represents the comparison of medians

Figure 3 : A. Receiver Operating Characteristics (ROC) curves of PCT, CRP1 and CRP2 in the whole population. B. Areas under the ROC curve of PCT, CRP1 and CRP2 in the whole population with confident interval 95%

Figure 4 : Nomogram of Bayes representing positive and negative post test probability according to pre test probability and positive and negative likelihood ratios in the whole and preterms populations. In the whole population pre test probability was 1.2% and post test probability was 28% in case of positive test and < 0.001% in case of negative test. Among preterms, pre test probability was 2% and post test probability was 30% in case of positive test and < 0.001% in case of negative test

487 Tab 1 : Baseline characteristics of patients suspect of infection.

Characteristic	All newborns (n=2151)		Full-terms (n=1339)		Preterms (<37 GW) (n=812)	
Mean Gestational age (SD) (weeks)	36.9	(3.8)	39.4	(1.2)	32.7	(2.9)
<28 weeks	52	(2.4)				
28-32 weeks	248	(11.5)				
32-36 weeks	512	(23.8)				
>36 weeks	1339	(62.3)				
Male sex	1141	(53)	684	(51)	446	(55)
Mean birth weight (SD) (g)	2783	(843.8)	329	(453.8)	2394	(649.2)
<1000 g	72	(3.3)	0	(0)	68	(8.4)
1001-1500 g	151	(7)	0	(0)	149	(18.3)
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APGAR score <7 at 5 min	61	(2.8)	14	(1)	47	(5.8)
pH ,at birth –mean	7.24		7.23		7.26	
<7.09	91	(4.2)	61	(4.6)	30	(3.7)
7.09-7.14	118	(5.5)	94	(7)	24	(3)
>7.14	1945	(90.4)	1187	(88.6)	758	(93.3)
Intrauterine growth retardation	136	(6.3)	28	(2.1)	108	(13.3)
Death before discharge from the hospital	28	(1.3)	3	(0.2)	25	(3.1)
Multiple pregnancy	281	(13.1)	17	(1.3)	264	(32.5)
Infection	26	(1.2)	9	(0.7)	17	(2.1)
Certain	3	(0.1)	1	(0.1)	2	(0.2)
Probable	23	(1.1)	8	(0.6)	15	(1.8)

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Table 2. Diagnosis values of PCT according to the groups of term.

Whole population	Whole population		Preterms		Fullterms	
Sensitivity	0.92	(0.75 - 0.98)	1	(0.82 - 1)	0.78	(0.45 - 0.94)
Specificity	0.97	(0.96 - 0.98)	0.95	(0.93 - 0.96)	0.98	(0.97 - 0.99)
Negative predictive value	0.99	(0.99 - 0.99)	1	(0.99 - 1)	0.99	(0.99 - 1)
Positive predictive value	0.28	(0.2 - 0.36)	0.3	(0.3 - 0.31)	0.23	(0.18 - 0.28)
Positive likelihood ratio	32	(24 - 41)	20	(14 - 27)	45	(26 - 77)
Negative likelihood ratio	0.08	(0.02 - 0.3)	0	(0 - 0.45)	0.23	(0.07 - 0.8)

Values are percentages (95% CI)

494 Table 3 : Analysis of factors related with infection diagnosis by univariate
495 analysis and multivariate analysis by logistic regression in the whole population.

Parameters	Crude OR (95% CI)	p	Adjusted OR (95% CI)	p
Chorioamnionitis	25.5 (10.6 - 61.3)	<0.001	8.3 (2.3 - 29.8)	0.001
Intrapartum fever	1.8 (0.8 - 2.3)	0.07	1.12 (0.5 - 3.1)	0.2
Spontaneous delivery < 37 GW	2.6 (0.6 - 10.9)	0.2	1 (0.9 - 1.1)	0.9
Length of membrane rupture > 12h	2.4 (1 - 5.5)	0.04	1.16 (0.4 - 3.7)	0.8
Maternal colonization with *streptococcus B*	0.84 (0.2 - 2.94)	0.5	0.8 (0.2 - 3.9)	0.8
Fetal asphyxia	13.4 (4.9 - 35.3)	<0.001	2.6 (0.7 - 10.2)	0.2
PCT > 0.6 ng/ml	399.3 (88.6 - 2501.9)	<0.001	257.6 (57.2 - 1159.1)	<0.001

496 Values are odds ratios with 95% confident interval.
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Table 4 : Analysis of factors increasing PCT value in cord blood by univariate analysis and multivariate analysis by logistic regression in the whole population.

Parameters	Crude OR (95% CI)	p	Adjusted OR (95% CI)	p
Gestational age <28 weeks	11.7 (5.5 - 25)	<0.001	6.2 (0.8 - 48.9)	0.06
Gestational age 28-32 weeks	4.9 (2.8 - 8.5)	<0.001	4.4 (1.2 - 16.2)	0.02
Gestational age 32-36 weeks	1.9 (1.1 - 3.3)	0.03	1.9 (0.8 - 4.6)	0.11
Birth weight<1000 g-	5.7 (2.7 - 11.9)	<0.001	0.7 (0.1 - 5.6)	0.69
Birth weight 1001-1500 g-	3.9 (2.1 - 7.3)	<0.001	0.6 (0.2 - 2.5)	0.57
Birth weight 1501-2500g-	1.7 (1 - 2.9)	0.4	0.9(0.3 - 2.3)	0.81
Intra uterine growth retardation	0.3 (0.1 - 1.4)	0.13	0.2(0.05 - 1.2)	0.08
Preeclampsia	0.4 (0.1 - 1.35)	0.15	0.3 (0.1 - 1.2)	0.07
pH < 7.10	2.4 (1.1 - 5.1)	0.02	2.9 (1.1 - 7.4)	0.04
pH 7.10–7.14	0.6 (0.2 - 2.1)	0.4	0.6 (0.1 - 2.7)	0.59
Apgar <7 at 5 min	8.1 (4.3 - 15.3)	<0.001	2.1 (0.8 - 5.6)	0.07
Multiple pregnancy	1.2 (0.6 - 2.2)	0.6	0.6 (0.3 - 1.3)	0.16
Infection	393.5 (91 - 1701.5)	<0.001	304.5 (63.7 - 1456.3)	<0.001
Prenatal corticosteroids exposure	2.9 (1.9-4.6)	<0.001	1.8 (0.8-3.9)	0.12

Values are odds ratios with 95% confident interval.

Legends

Figure 1 : Study flow diagram

Figure 2 : Box-and whisker plot showing PCT concentration according to the infectious status. PCT level is expressed in ng/ml. Box represent first and third quartiles, horizontal lines represent medians with the corresponding numerical value, whiskers give 10/90 percentiles. Open circle represents oulier. Dotted line represents the cut-off of 0,6 ng/ml. p represents the comparison of medians

Figure 3 : A. Receiver Operating Characteristics (ROC) curves of PCT, CRP1 and CRP2 in the whole population. B. Areas under the ROC curve of PCT, CRP1 and CRP2 in the whole population with confident interval 95%

Figure 4 : Nomogram of Bayes representing positive and negative post test probability according to pre test probability and positive and negative likelihood ratios in the whole and preterms populations. In the whole population pre test probability was 1.2% and post test probability was 28% in case of positive test and < 0.001% in case of negative test. Among preterms, pre test probability was 2% and post test probability was 30% in case of positive test and < 0.001% in case of negative test

527 Tab 1 : Baseline characteristics of patients suspect of infection.

Characteristic	All newborns (n=2151)		Full-terms (n=1339)		Preterms (<37 GW) (n=812)	
Mean Gestational age (SD) (weeks)	36.9	(3.8)	39.4	(1.2)	32.7	(2.9)
<28 weeks	52	(2.4)				
28-32 weeks	248	(11.5)				
32-36 weeks	512	(23.8)				
>36 weeks	1339	(62.3)				
Male sex	1141	(53)	684	(51)	446	(55)
Mean birth weight (SD) (g)	2783	(843.8)	329	(453.8)	2394	(649.2)
<1000 g	72	(3.3)	0	(0)	68	(8.4)
1001-1500 g	151	(7)	0	(0)	149	(18.3)
1501-2500g	472	(21.9)	59	(4.4)	415	(51.1)
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Prenatal corticosteroids exposure	423	(19.7)	19	(1.4)	404	(49.8)
Gastric fluid colonisation	312	(14.5)	208	(15.5)	102	(12.6)
Membrane rupture >24h	578	(26.9)	348	(26)	232	(28.6)
Caesarean	691	(32.1)	276	(20.6)	414	(51)
Antenatal antibiotics exposure	699	(32.5)	409	(30.5)	290	(35.7)
Chorioamnionitis	82	(3.8)	10	(0.7)	72	(8.8)
Pre-eclampsia	164	(7.6)	42	(3.1)	122	(15)
APGAR score <7 at 5 min	61	(2.8)	14	(1)	47	(5.8)
pH ,at birth –mean	7.24		7.23		7.26	
<7.09	91	(4.2)	61	(4.6)	30	(3.7)
7.09-7.14	118	(5.5)	94	(7)	24	(3)
>7.14	1945	(90.4)	1187	(88.6)	758	(93.3)
Intrauterine growth retardation	136	(6.3)	28	(2.1)	108	(13.3)
Death before discharge from the hospital	28	(1.3)	3	(0.2)	25	(3.1)
Multiple pregnancy	281	(13.1)	17	(1.3)	264	(32.5)
Infection	26	(1.2)	9	(0.7)	17	(2.1)
Certain	3	(0.1)	1	(0.1)	2	(0.2)
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Whole population	Whole population		Preterms		Fullterms	
Sensitivity	0.92	(0.75 - 0.98)	1	(0.82 - 1)	0.78	(0.45 - 0.94)
Specificity	0.97	(0.96 - 0.98)	0.95	(0.93 - 0.96)	0.98	(0.97 - 0.99)
Negative predictive value	0.99	(0.99 - 0.99)	1	(0.99 - 1)	0.99	(0.99 - 1)
Positive predictive value	0.28	(0.2 - 0.36)	0.3	(0.3 - 0.31)	0.23	(0.18 - 0.28)
Positive likelihood ratio	32	(24 - 41)	20	(14 - 27)	45	(26 - 77)
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Parameters	Crude OR (95% CI)	p	Adjusted OR (95% CI)	p
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Intrapartum fever	1.8 (0.8 - 2.3)	0.07	1.12 (0.5 - 3.1)	0.2
Spontaneous delivery < 37 GW	2.6 (0.6 - 10.9)	0.2	1 (0.9 - 1.1)	0.9
Length of membrane rupture > 12h	2.4 (1 - 5.5)	0.04	1.16 (0.4 - 3.7)	0.8
Maternal colonization with *streptococcus B*	0.84 (0.2 - 2.94)	0.5	0.8 (0.2 - 3.9)	0.8
Fetal asphyxia	13.4 (4.9 - 35.3)	<0.001	2.6 (0.7 - 10.2)	0.2
PCT > 0.6 ng/ml	399.3 (88.6 - 2501.9)	<0.001	257.6 (57.2 - 1159.1)	<0.001

536 Values are odds ratios with 95% confident interval.
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Parameters	Crude OR (95% CI)	p	Adjusted OR (95% CI)	p
Gestational age <28 weeks	11.7 (5.5 - 25)	<0.001	6.2 (0.8 - 48.9)	0.06
Gestational age 28-32 weeks	4.9 (2.8 - 8.5)	<0.001	4.4 (1.2 - 16.2)	0.02
Gestational age 32-36 weeks	1.9 (1.1 - 3.3)	0.03	1.9 (0.8 - 4.6)	0.11
Birth weight<1000 g-	5.7 (2.7 - 11.9)	<0.001	0.7 (0.1 - 5.6)	0.69
Birth weight 1001-1500 g-	3.9 (2.1 - 7.3)	<0.001	0.6 (0.2 - 2.5)	0.57
Birth weight 1501-2500g-	1.7 (1 - 2.9)	0.4	0.9(0.3 - 2.3)	0.81
Intra uterine growth retardation	0.3 (0.1 - 1.4)	0.13	0.2(0.05 - 1.2)	0.08
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pH < 7.10	2.4 (1.1 - 5.1)	0.02	2.9 (1.1 - 7.4)	0.04
pH 7.10–7.14	0.6 (0.2 - 2.1)	0.4	0.6 (0.1 - 2.7)	0.59
Apgar <7 at 5 min	8.1 (4.3 - 15.3)	<0.001	2.1 (0.8 - 5.6)	0.07
Multiple pregnancy	1.2 (0.6 - 2.2)	0.6	0.6 (0.3 - 1.3)	0.16
Infection	393.5 (91 - 1701.5)	<0.001	304.5 (63.7 - 1456.3)	<0.001
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Apgar <7 at 5 min	8.1 (4.3 - 15.3)	<0.001	2.1 (0.8 - 5.6)	0.07
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