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Non-invasive prediction of intra-amniotic inflammation in women with preterm labor

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KEYWORDS: cervical length; gestational age; intra-amniotic inflammation; preterm labor; white blood cell count

ABSTRACT

Objective *To develop a model based on non-invasive variables to predict the probability of intra-amniotic inflammation in women with preterm labor and intact membranes.*

Methods *Transvaginal ultrasonography and digital examination for the assessment of cervical length and cervical dilatation were performed, and maternal blood was collected for the determination of C-reactive protein and white blood cell (WBC) count immediately after amniocentesis in 153 consecutive women with preterm labor. Amniotic fluid obtained by amniocentesis was cultured for aerobic and anaerobic bacteria and mycoplasmas, and the WBC was determined. Intra-amniotic inflammation was defined as an elevated amniotic fluid interleukin-6 concentration (> 2.6 ng/mL). Receiver–operating characteristics (ROC) curves and logistic regression analysis were used for statistical analysis.*

Results *The prevalence of a positive amniotic fluid culture was 7.2% (11/153) and the prevalence of intra-amniotic inflammation was 19.6% (30/153). The final logistic regression model was based on non-invasive clinical variables, including gestational age at assessment, cervical length and maternal blood WBC count, which were the best predictors of intra-amniotic inflammation. The model was shown to have an adequate goodness of fit ($P = 0.754$), and the area under the ROC curve was 0.724, indicating reasonably good discrimination.*

Conclusion *In women with preterm labor and intact membranes, the risk for intra-amniotic inflammation can be predicted non-invasively with a risk score based on gestational age, cervical length and maternal blood WBC count. Copyright © 2010 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

A growing body of evidence indicates that women in preterm labor with intra-amniotic inflammation, but not microbial invasion of the amniotic cavity, have a similar outcome to those with microbial invasion of the amniotic cavity[1,2]. Therefore, from a practical point of view, an early and accurate detection of intra-amniotic inflammation may be more important than that of microbial invasion of the amniotic cavity in the management of women with preterm labor. The antenatal detection of intra-amniotic inflammation requires laboratory studies of white blood cells (WBC) and inflammatory markers in amniotic fluid (AF) obtained by amniocentesis, such as AF cytokine determination[1–4]. However, amniocentesis is an invasive method[5], although it is generally considered a relatively simple and safe procedure when performed by an experienced physician. Consequently, alternative approaches for the rapid and non-invasive identification of intra-amniotic inflammation are needed for women with preterm labor.

Transvaginal ultrasonography has been widely accepted as a non-invasive and objective method for the evaluation of cervical status in women with preterm labor[6,7]. Several studies have shown a significant relationship between a short cervix detected by transvaginal ultrasound, microbial invasion of the amniotic cavity, and impending delivery in women with preterm labor[8–10], while measurement of C-reactive protein or the WBC count in maternal blood has been shown to be a clinically useful biochemical non-invasive approach for discriminating women in preterm labor who are at high risk for microbial invasion of the amniotic cavity and failed tocolysis[11–13]. However, to date, there has been a paucity of data regarding non-invasive clinical and sonographic parameters, individually or in combination, as a clinically

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useful method for identifying intra-amniotic inflammation in women with preterm labor. This information is clinically relevant, since it may decrease unnecessary invasive intervention (e.g. amniocentesis) for women in preterm labor and non-invasively identify patients who may benefit from close surveillance and more aggressive treatment, such as administration of antibiotics or corticosteroid. The purpose of this study was to develop a model based on non-invasive clinical parameters to predict the probability of intra-amniotic inflammation in women with preterm labor and intact membranes.

PATIENTS AND METHODS

The study population consisted of consecutive women admitted to Seoul National University Bundang Hospital between October 2005 and December 2009 with the diagnosis of preterm labor and intact membranes who met the following criteria: (1) singleton gestation; (2) transabdominal amniocentesis performed for microbiological studies of amniotic cavity; (3) cervical length measured at the time of amniocentesis; (4) maternal blood drawn immediately after amniocentesis for the determination of WBC and C-reactive protein concentrations; (5) a live fetus with a gestational age between 21 and 35 weeks; (6) cervical dilatation of ≤ 3 cm by digital examination; (7) no history of prior or subsequent cervical cerclage; and (8) absence of a major fetal congenital anomaly. The non-invasive clinical parameters that were collected at the time of enrollment included demographic variables (maternal age, parity and previous preterm delivery), gestational age at assessment, C-reactive protein concentration, maternal serum WBC count, cervical length assessed by transvaginal ultrasonography and cervical dilatation by digital examination. Clinical and laboratory data were collected prospectively. Amniocentesis for retrieval of AF and measurement of cervical length are recommended to all patients who are admitted with the diagnosis of preterm labor and intact membranes at our institution. The study was approved by the Institutional Review Board of the Seoul National University Bundang Hospital, and informed consent was obtained from all study subjects.

Patients were hydrated and if uterine contractions persisted, they were started on a regimen of intravenous tocolysis with ritodrine or magnesium sulfate after amniocentesis. Corticosteroids were administered between 24 and 34 weeks' gestation. Preterm labor was defined as the presence of regular uterine contractions with a frequency of at least two every 10 min and cervical change before 37 completed weeks of gestation that required hospitalization. Intra-amniotic infection was defined as a positive AF culture for microorganisms. Intra-amniotic inflammation was diagnosed by an amniotic fluid interleukin-6 (IL-6) concentration > 2.6 ng/mL, as previously reported[1].

Transabdominal amniocentesis was used to obtain AF under ultrasonographic guidance. Microbiological evaluation included cultures for aerobic and anaerobic bacteria and mycoplasmas (*Ureaplasma urealyticum* and *Mycoplasma hominis*), according to methods previously described[14]. An aliquot of AF was transferred to the hematology laboratory and examined in a hemocytometer chamber for the presence of WBCs. The absolute WBC count was calculated by multiplying the area examined by a factor of 10 per area and was expressed as the number of cells per cubic mm. AF that was not required for clinical assessment was centrifuged, and the supernatant liquid was aliquoted and stored at $-70°C$ until assayed. Samples were not subjected to freeze–thaw cycles before being assayed. IL-6 concentrations were measured by an enzyme-linked immunosorbent assay (human IL-6 DuoSet Kit; R&D Systems, Minneapolis, MN, USA). The range of the IL-6 standard curve is 7.8–600 pg/mL. The assay was carried out by strictly following the instructions provided by the manufacturer and all samples were measured in duplicate at the same time. The calculated intra- and interassay coefficients of variation were $< 10\%$. The maternal blood WBC count was determined using an automated hemocytometer (XE-2100; Sysmex, Tokyo, Japan). The C-reactive protein concentration was measured with a latex-enhanced turbidimetric immunoassay (Denka Seiken, Tokyo, Japan) using Toshiba 200FR (Toshiba, Tokyo, Japan).

Transvaginal ultrasonographic assessment of cervical length was performed by trained physicians immediately after amniocentesis using an Envisor ultrasound machine with a 6.0-MHz transducer (Philips Medical Systems, Eindhoven, The Netherlands). The method used for measurements of cervical length has been previously described[15]. Three measurements were performed and the shortest distance was taken as the cervical length.

Univariate analysis was conducted with the Student's *t*-test, Mann–Whitney *U*-test, or chi-square test. The Shapiro–Wilk and Kolmogorov–Smirnov tests were used to test for normal distribution of data and logistic regression analysis was used to investigate the regression relationship between various predictors and intra-amniotic inflammation. To develop a non-invasive model for the prediction of intra-amniotic inflammation, non-invasive clinical parameters that presented a significant correlation or a tendency towards an association with intra-amniotic inflammation in univariate analysis ($P < 0.25$) were subjected to stepwise backward logistic regression analysis. The linearity of continuous variables was assessed using fractional polynomials[16]. The goodness-of-fit of a logistic regression model was assessed by the Hosmer–Lemeshow test. Factors included in the final logistic regression model were used to set up the scores for the probability of intra-amniotic inflammation. Using the constant of the logistic regression analysis and the beta-coefficients for the identified predictive factors in the final risk scoring model, the individual probability of having an intra-amniotic inflammation for each woman in preterm labor was calculated. Discriminatory ability of this model was measured by the area under the receiver–operating characteristics (ROC) curve[17]. The model's value that maximized the sum of the sensitivity and specificity

and was able to minimize the misclassification rate was considered the best cut-off. A univariate *Z*-score test was used to estimate the statistical significance of the difference in the area under the curve between the AF-WBC count and the final risk scoring model[18]. All reported *P*-values are two-sided, and $P < 0.05$ was considered statistically significant. SPSS 12.0.1 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

RESULTS

During the study period, 165 consecutive women with preterm labor were recruited. Of the 165 women, AF was not available in seven women for IL-6 determination (six women with a negative AF culture and one woman with a positive AF culture) and five had a history of cervical cerclage placement during the index pregnancy. These women were excluded from the study, leaving 153 women suitable for evaluation. The gestational age of the women included in our study at the time of amniocentesis ranged from $21 + 2$ to $35 + 0$ weeks' gestation. The overall rate of intra-amniotic inflammation was 19.6% (30/153) and the prevalence of a positive AF culture was 7.2% (11/153). The microorganisms isolated from the amniotic cavity included *Ureaplasma urealyticum* ($n = 10$) and *Mycoplasma hominis* ($n = 9$), with both found in eight cases. Intra-amniotic inflammation was present in all cases with a positive AF culture and intra-amniotic inflammation with a negative AF culture occurred in 13.4% of samples (19/142).

Table 1 shows the demographic and clinical characteristics of the women according to the presence or absence of intra-amniotic inflammation. Women with intra-amniotic inflammation had a significantly lower mean gestational age at assessment and at delivery, a lower mean cervical length, and a higher mean maternal serum C-reactive protein concentration and WBC count than did those without intra-amniotic inflammation. However, there were no significant differences in the mean maternal age, nulliparity, prevalence of previous spontaneous preterm delivery and mean cervical dilatation between the two groups. The mean AF-WBC count and the proportion of positive AF cultures were significantly higher in women with intra-amniotic inflammation than in those without intra-amniotic inflammation.

To identify the final non-invasive predictors of intra-amniotic inflammation, we performed multivariate analysis using a stepwise logistic regression model. After the exclusion of variables (i.e. AF-WBC count and AF culture results) using invasive amniocentesis, four variables with a significant correlation or a tendency towards an association with intra-amniotic inflammation based on univariate analysis ($P < 0.25$) were entered into the multivariate model (gestational age at assessment, sonographic cervical length, maternal serum C-reactive protein and maternal blood WBC count). The final variables retained in the non-invasive model were gestational age at assessment, cervical length and maternal blood WBC count (Table 2). There were no statistically significant interactions between the predictors selected in the multivariate model. Because fractional polynomial analysis yielded significant linear functions for these three predictors, we used these as continuous variables. The formula that was generated to predict intra-amniotic inflammation is: $Y = \log_e(Z) = 3.375 - (0.180 \times \text{gestational age}) + (0.130 \times \text{WBC count}) - (0.399 \times \text{cervical length})$, where gestational age is given in weeks, WBC count in thousands of cells per mm^3 and cervical length in cm. $Z = e^y$ and risk (%) $= (Z/(1 + Z)) \times 100$.

The discriminatory ability of this model was 0.724 (95% confidence interval (CI), 0.622–0.826), suggesting a reasonably good discrimination between women with and without intra-amniotic inflammation. The Hosmer–Lemeshow test showed an adequate fit of the model studied ($P = 0.754$).

The sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratios of the different cut-offs of probability for intra-amniotic inflammation using the formula with the three non-invasive parameters are shown in Table 3. A risk score of 20% for a given woman in preterm labor was

Table 1 Demographic and clinical characteristics of the study population according to presence or absence of intra-amniotic inflammation

Characteristic	*Intra-amniotic inflammation*		P
	Absent (n = *123*)	*Present* (n = *30*)	
Maternal age (years)	31.0 ± 4.1	31.3 ± 4.5	0.753
Nulliparous	77 (62.6)	17 (56.7)	0.676
Previous spontaneous preterm birth (at < 37 weeks)	10 (8.1)	2 (6.7)	1.0
Gestational age at assessment (weeks)	30.7 ± 3.2	28.7 ± 3.6	0.004
Gestational age at delivery (weeks)	36.6 ± 3.1	31.3 ± 4.9	< 0.0001
Cervical length by ultrasound (mm)	26.8 ± 12.0	20.8 ± 11.0	0.014
Cervical dilatation by digital examination (mm)	7.1 ± 9	6.7 ± 10	0.792
White blood cell count (cells/mm^3)	10 755 ± 3258	12 474 ± 3135	0.010
C-reactive protein (mg/dL)	0.9 ± 1.5	1.8 ± 1.9	0.013
Amniotic fluid white blood cell count (cells/mm^3)	8.9 ± 31.0	916.1 ± 1360.9	0.001
Positive amniotic fluid culture	0	11 (36.7)	< 0.0001

Values are given as mean ± SD or *n* (%). Intra-amniotic inflammation defined as interleukin-6 level > 2.6 ng/mL.

Table 2 Regression coefficients, odds ratios, and 95% CIs of the final model to predict probability of having intra-amniotic inflammation

Predictor	*Beta-coefficient*	*SE*	*Odds ratio (95% CI)*	P
Gestational age at assessment (weeks)	−0.180	0.063	0.836 (0.739–0.945)	0.004
While blood cell count (thousand/mm^3)	0.130	0.063	1.139 (1.007–1.289)	0.038
Cervical length (cm)	−0.399	0.186	0.671 (0.466–0.965)	0.032
Constant	3.375	2.063	29.230	0.102

Table 3 Sensitivity, specificity, positive (PPV) and negative (NPV) predictive values and likelihood ratios (LR) of the different cut-offs of probability for intra-amniotic inflammation predicted from the final model using three non-invasive parameters

Predicted probability	*Screened positive* (n *(%))*	*Sensitivity (%)*	*Specificity (%)*	*PPV (%)*	*NPV (%)*	*LR+*	*LR−*
≥ 0.1	103 (67.3)	83.3	36.6	24.3	90.0	1.31	0.46
≥ 0.2	55 (35.9)	66.7	71.5	36.4	89.8	2.34	0.47
≥ 0.3	36 (23.5)	46.7	82.1	38.9	86.3	2.61	0.65
≥ 0.4	13 (8.5)	20.0	94.3	46.2	82.9	3.51	0.85
≥ 0.5	7 (4.6)	16.7	98.4	71.4	82.9	10.44	0.85

identified as being the optimal threshold for sensitivity and specificity, and a high proportion of women classified correctly. Thirty-six percent of the women had an estimated probability for intra-amniotic inflammation ≥ 0.2, and 36% of these women were diagnosed with intra-amniotic inflammation. Moreover, this group included 67% of all women who had intra-amniotic inflammation.

Figure 1 displays ROC curves comparing the predictions provided by the AF-WBC count and the non-invasive model for the risk of intra-amniotic inflammation. The area under the ROC curve for the AF-WBC count was slightly larger than that for the non-invasive model; however, these results were determined not to be statistically significant for a 95% confidence level (0.826 (95% CI, 0.724–0.928) vs. 0.724 (95% CI, 0.622–0.826), respectively; $P = 0.165$). Nonetheless, in comparison with each of the three variables retained in the non-invasive model, ROC curve analysis demonstrated that an AF-WBC count was superior to any other parameter in the prediction of intra-amniotic inflammation (gestational age at assessment, cervical length and maternal blood WBC count; $P < 0.05$ for each). In comparison, the area under the ROC curve for the non-invasive model was significantly higher than that for cervical length ($P = 0.033$) but not significantly higher than that for maternal WBC count ($P = 0.342$) and gestational age at assessment ($P = 0.259$).

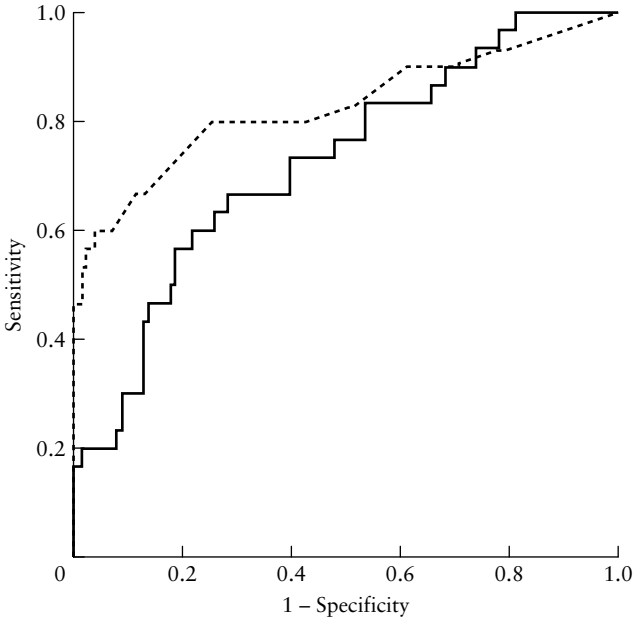

Figure 1 Receiver–operating characteristics (ROC) curves comparing the predictions provided by the amniotic fluid (AF) white blood cell (WBC) count (- - - - -) and the non-invasive model (——) for the risk of intra-amniotic inflammation. There was no statistically significant difference between the areas under the ROC curves (AUC) of AF-WBC count and the non-invasive model (AUC for AF-WBC, 0.826; SE 0.052, $P < 0.0001$; AUC for non-invasive model, 0.724; SE 0.052, $P < 0.0001$; $P = 0.165$ for difference between the two ROC curves).

DISCUSSION

The results of our study clearly demonstrate that the risk of intra-amniotic inflammation among women with preterm labor and intact membranes can be predicted by three non-invasive clinical parameters (gestational age at assessment, cervical length and maternal WBC count). Moreover, the model obtained by combining these parameters has reasonably good discriminatory power and may be useful for selecting the subset of women with preterm labor and intact membranes requiring invasive amniocentesis.

The novelty of our approach for identifying intra-amniotic inflammation is the use of combinations of non-invasive parameters, by means of which we can obtain a higher discrimination power than is possible with any single parameter. The area under the curve from ROC-curve analysis ranged from 0.638 to 0.676 for each

of the three parameters individually; the combination of these parameters improved the diagnostic accuracy to an area under the curve of 0.724, although the improvement reached statistical significance only in the comparison of cervical length. These findings support the notion that a single predictive model that combines clinical, laboratory and ultrasound parameters is useful for providing a clinically meaningful non-invasive prediction of intra-amniotic inflammation in women with preterm labor.

From a practical point of view, we propose that screening for intra-amniotic inflammation should begin with this non-invasive model and that those women shown to be in the high-risk group could then be considered for amniocentesis to obtain a quantitative AF-IL-6 level, which is a more accurate prediction of intra-amniotic inflammation. Based on our suggested risk-score threshold (≥ 0.2), the use of this model would lead to safely avoiding amniocentesis in 89% of women with clinical and laboratory data indicating the absence of an intra-amniotic inflammation and reduce the number of amniocenteses by approximately two-thirds (64%). However, this risk-score threshold is not fixed, and can vary according to the patient's or obstetrician's tolerance of risk. Some may prefer to select a lower risk threshold to ensure that women truly at high risk for intra-amniotic inflammation are not missed, whereas others may prefer to choose a higher threshold value to minimize the false-positive error rate. For example, as shown in Table 3, a lower score threshold (e.g. ≥ 0.1) would have the greatest sensitivity (83.3%) and negative predictive value (90%), which would make this model an excellent tool for the identification of women at low risk of intra-amniotic inflammation and in whom amniocentesis may be avoided, while reducing the number of amniocenteses by 33%. By contrast, a higher score threshold (e.g. ≥ 0.5) would minimize the false-positive rate (1.6%), but increase the likelihood of a false-negative result (83.3%) and the corresponding risk region would comprise only 4.6% of the population. Furthermore, this cut-off threshold has a limited positive predictive value of 71.4%, which is unacceptably low for a diagnostic test.

Only two published studies have reported a significant association between intra-amniotic inflammation and a short cervix in women with preterm labor and intact membranes[19,20]. In the current study this association was confirmed by means of univariate analysis, and remained significant even after adjustment for gestational age at the time of examination. Similar observations have been reported in the setting of intra-amniotic infection in women with preterm labor[8–10]. The proposed mechanism for these observations is that microorganisms that gain access to the intrauterine cavity stimulate the production of pro-inflammatory cytokines and inflammatory mediators, and cause cervical shortening secondarily. Alternatively, it could be that an already shortened cervix predisposes women to ascending bacterial infections from the vagina and cervix, and then induces activation of cytokines.

Our observation that an elevated WBC count or C-reactive protein concentration in the maternal blood is associated with intra-amniotic inflammation in women with preterm labor was not unexpected because several studies have demonstrated that an AF-IL-6 determination is an excellent predictor of histologic chorioamnionitis reflecting a maternal inflammatory response[14,21]. Indeed, an inflammatory process during the course of an ascending intrauterine infection is generally regarded as a continuum of maternal and fetal inflammatory responses. During the early stage, localized inflammation confined to the chorion-decidua, which represents the maternal host response, releases various cytokines in the maternal circulation, and these, in turn, lead to leukocytosis and C-reactive protein production[22,23]. On the other hand, the later stage involves microbial invasion of the amniotic cavity through the amnion, where microorganisms can also stimulate the production of inflammatory mediators by resident macrophages and other host cells, and eventually leads to a fetal systemic inflammatory response[23]. Therefore, given that the intra-amniotic inflammatory response is a part of the advanced stage of an ascending intrauterine infection and is solely of fetal origin[23,24], our observations strongly suggest that many women with a marked rise in inflammatory markers in peripheral blood may already have a fetal systemic inflammatory response mediated by cytokines. Further studies are needed to focus on whether maternal blood tests, such as the WBC count and C-reactive protein level reflecting the maternal inflammatory response, are of value in predicting a fetal systemic inflammatory response in women with preterm labor. Our finding that the earlier the gestational age at which the woman presented, the more likely that an intra-amniotic inflammation was present, is in agreement with previous studies[1,20]. In addition, other investigators have also reported an inverse relationship between gestational age at presentation and microbial colonization of the AF or chorioamnion in women with preterm labor[4,25].

The present study had several limitations. First, our cohort consisted of women from one hospital; therefore, our non-invasive model may not be generalizable to other populations of women and consequently a prospective validation in another study population is needed to establish the actual validity. Second, the model did not include some previously reported important non-invasive predictors, such as determination of cervical fluid cytokines[10,26,27]. Third, in the present study the prevalence of a positive AF culture was 7.2% and the prevalence of intra-amniotic inflammation was 19.6%, values that are lower than those reported by other investigators (range for a positive AF culture, 8.7–33.8%; range for intra-amniotic inflammation, 30–39%)[5,7,28]. However, the prevalence of intra-amniotic inflammation may be affected by how it is defined, the gestational age at which the amniocentesis was performed and the threshold required to perform an amniocentesis in women with preterm labor at different centers.

In conclusion, we have demonstrated that a model based on easily obtainable, non-invasive parameters in the setting of preterm labor with intact membranes can identify women at high risk for intra-amniotic inflammation. This model can serve as an important tool for predicting individual intra-amniotic inflammation risk probabilities for obstetricians and may enhance patient care by targeting aggressive therapy, including amniocentesis and antibiotic/anti-inflammatory treatment, to high-risk groups.

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