AOGS
Acta Obstetricia et Gynecologica Scandinavica

AOGS ORIGINAL RESEARCH ARTICLE

Neutrophil elastase in amniotic fluid as a predictor of preterm birth after emergent cervical cerclage

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Key words
Amniotic fluid, cervical cerclage, glucose, neutrophil elastase, pregnancy, premature birth

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Conflicts of interest
The authors have stated explicitly that there are no conflicts of interest in connection with this article.

Please cite this article as: Hatakeyama Y, Miura H, Sato A, Onodera Y, Sato N, Shimizu D, et al. Neutrophil elastase in amniotic fluid as a predictor of preterm birth after emergent cervical cerclage. Acta Obstet Gynecol Scand 2016; 95:1136–1142.

Received: 16 March 2016
Accepted: 19 May 2016

DOI: 10.1111/aogs.12928

Abstract

Introduction. The aim of this study was to investigate neutrophil elastase (NE) in amniotic fluid as a potential marker for predicting pregnancy continuation. *Material and methods.* We enrolled 34 pregnant women with bulging fetal membrane during the second trimester who underwent emergent cerclage after confirming the absence of intrauterine infection (amniotic fluid glucose ≥15 mg/dL). Amniotic fluid NE levels were compared between women who completed and did not complete 30, 34, and 36 weeks of gestation, and the optimal cut-off value for predicting pregnancy continuation was estimated. Moreover, the differences in the duration of continued pregnancy were compared between women with NE levels above and below the optimal cut-off value. *Results.* The optimal cut-off value for NE in amniotic fluid that predicted pregnancy continuation beyond 30, 34, and 36 weeks of gestation was 180 ng/mL; this cut-off value had a sensitivity, specificity, positive predictive value, and negative predictive value of 84.0, 77.8, 91.3, and 63.7% beyond 30 weeks of gestation; 87.5, 80.0, 91.5, and 72.3% beyond 34 weeks of gestation; and 85.0, 71.4, 80.9, and 76.9% beyond 36 weeks of gestation, respectively. The duration of continued pregnancy from emergent cerclage to delivery was significantly longer in women with amniotic fluid NE <180 ng/mL (95.1 ± 5.4 days) than in women with amniotic fluid NE ≥180 ng/mL (44.8 ± 14.3 days). *Conclusion.* The NE levels in amniotic fluid may serve as a useful marker for predicting the duration of continued pregnancy after cervical cerclage.

Abbreviations: IL-6, interleukin-6; NE, neutrophil elastase.

Introduction

Cervical cerclage is used to prolong gestation in pregnant women with possible cervical insufficiency, and is classified into three types: elective cerclage, performed at 12–14 weeks of gestation in women with a history of miscarriage in the second trimester; urgent cerclage, performed before 24 weeks of gestation in women with no clinical symptoms, but with cervical shortening or

Key Message

Neutrophil elastase levels in amniotic fluid may be a useful marker for predicting the duration of continued pregnancy after emergent cervical cerclage in pregnant women with bulging fetal membrane.

funneling of the membranes into the internal cervical os according to transvaginal ultrasonography; and rescue cerclage, performed for women with symptoms, such as vaginal bleeding and pelvic pressure, who also exhibit obvious cervical dilatation and bulging of the fetal membranes on physical examination (1).

Urgent and rescue cerclage are both known to be effective as therapeutic cerclage (1–4); however, therapeutic cerclage has been reported to lead to preterm premature rupture of membranes or chorioamnionitis in some cases, resulting in miscarriage or premature birth (5–8). Therefore, to ensure the effectiveness of therapeutic cerclage, it is necessary to assess whether intrauterine inflammation is present preoperatively.

Amniotic inflammation can be directly evaluated by measuring the levels of interleukin-6 (IL-6), glucose, and neutrophil elastase (NE) in the amniotic fluid (9–19). Neutrophil elastase is a serine protease, which decomposes most components of the extracellular matrix. It is released from leukocytes, which migrate from the chorionic membrane in the inflammatory stage at a rate proportional to chorioamnionitis severity (11). We observed that women with threatened premature birth who were diagnosed with chorioamnionitis have an increased NE level in their amniotic fluid at each stage of chorioamnionitis based on Blanc's criteria, which is correlated with the severity of intrauterine inflammation (9).

We hypothesized that the amniotic fluid NE level would reflect the severity of intrauterine infection and predict premature birth after therapeutic cervical cerclage. Here, we aimed to assess the effectiveness of using the amniotic fluid NE level for predicting the duration of continued pregnancy after therapeutic cervical cerclage.

Material and methods

In this retrospective study, we enrolled women with a gestational age between 19 and 26 weeks who were admitted to the Akita Red Cross Hospital, Japan between January 2000 and August 2011 for bulging fetal membranes, and were treated with therapeutic cervical cerclage. All women underwent transabdominal amniocentesis using a thin needle (25-gauge) upon admission. Bacteriological culture was performed using an amniotic fluid sample. We excluded women with preterm premature rupture of membranes, defined as the leakage of amniotic fluid from the vagina, which was confirmed using the Nitrazine paper reaction. Moreover, women with fetal anomalies and intrauterine fetal death were excluded. The indications for therapeutic cerclage included only mild or no uterine contractions, and the absence of progressive cervical ripening and intrauterine infection, as evidenced by an amniotic fluid glucose concentration of ≥15 mg/dL (17,20). All women underwent a Shirodkar or McDonald cerclage. Women were treated postoperatively with intravenous antibiotic administration for 3 days, including the day of the procedure, and with antibiotic vaginal tablets for 6 days. All women provided informed consent for amniocentesis and cervical cerclage, and for conducting this study.

The study protocol was approved by the Ethics Committee of Akita University Graduate School of Medicine and Faculty of Medicine on 25 June 2015 (reference number 1337). The women's NE levels were also measured at Akita University. In brief, the women's amniotic fluid was centrifuged (at 1000 *g* for 10 min), and the supernatant was frozen at −30°C for subsequent NE measurements. The NE levels were measured according to the manufacturer's instructions using an Inotech Elastase kit (Sanwa Kagaku Kenkyusho Co., Nagoya, Japan) with a Model 680 microplate reader (Bio-Rad, Hercules, CA, USA), based on the latex immunoassay technique, and expressed as mean ± standard deviations. The sensitivity, expressed as minimal detectable dose, was 0.1 μg/min, according to the manufacturer's data. The intra-assay and inter-assay coefficients of variation of five amniotic fluid samples were previously calculated to be 0.8–2.5 and 1.0–1.5%, respectively.

Based on the three study end-points (30, 34, and 36 weeks of gestation), women were divided into those who continued pregnancy beyond the study end-point and those who had a premature birth before the study end-point. The NE levels were compared between these groups. Furthermore, we evaluated the differences in the duration of continued pregnancy between women with NE levels above and below the optimal cut-off value.

Statistical analysis

A Mann–Whitney *U*-test was used to compare the differences in NE values between groups; a p-value of <0.05 indicated statistical significance. Receiver operating characteristic curves were generated for NE concentrations in amniotic fluid, and the optimal NE cut-off values for continuing pregnancy beyond these three gestational ages were investigated. Afterwards, the durations of continued pregnancy from cervical cerclage to delivery were expressed as Kaplan–Meier curves for women with amniotic fluid NE levels above and below the cut-off values; a log-rank test was then used to determine significant differences between those with NE levels above and below the cut-off values. JMP® 12 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis.

Results

Amniocentesis was performed in 34 Japanese women to assess the intrauterine infection status and to establish a

treatment plan. We did not observe any women with culture-positive amniotic fluid or clinical chorioamnionitis (i.e. a temperature elevation to 38°C, uterine tenderness, malodorous vaginal discharge, fetal tachycardia, and maternal leukocytosis). The characteristics of the women are shown in Table 1. The proportion of women who continued pregnancy beyond 30, 34, and 36 weeks of gestation was 73.5% (25/34), 70.1% (24/34), and 58.8% (20/34), respectively. The receiver operating characteristic curves for the NE levels in amniotic fluid that are predictive of continuing pregnancy beyond 30, 34, and 36 weeks of gestation are shown in Figure 1. Based on these receiver operating characteristic curves, the cut-off value for the NE levels in amniotic fluid for continuing pregnancy beyond 30, 34, and 36 weeks of gestation was found to be 180 ng/mL; the corresponding areas under the curve were 0.844, 0.875, and 0.814 beyond 30, 34, and 36 weeks of gestation, respectively. The cut-off value of 180 ng/mL for predicting the continuation of pregnancy had a sensitivity, specificity, positive predictive value, negative predictive value, and odds ratio of 84.0, 77.8, 91.3, 63.7%, and 18.4 (95% CI 2.7–122.9) beyond 30 weeks of gestation; 87.5, 80.0, 91.5, 72.3%, and 28.0 (95% CI 3.9–199.9) beyond 34 weeks of gestation; and 85.0, 71.4, 80.9, 76.9%, and 14.2 (95% CI 2.6–76.7) beyond 36 weeks of gestation, respectively. The rates of premature birth among women before 30, 34, and 36 weeks of gestation were 26.5% (9/34), 29.4% (10/34), and 41.1% (14/34), respectively in the present study; after excluding 12 women with NE concentrations higher than the optimal cut-off value, the corresponding rates of premature birth were 13.6% (3/22), 13.6% (3/22), and 27.2% (6/22), respectively. Therefore, it appears that addition NE level in amniotic fluid to the indication for cerclage would have reduced the rate of premature birth at 30, 34, and 36 weeks of gestation by approximately 15% (Table 2). The Kaplan–Meier curves of the duration of continued pregnancy (from cerclage to delivery) in women with NE concentrations <180 ng/mL and ≥180 ng/mL are shown in Figure 2. The mean duration of continued pregnancy among women with NE concentrations <180 ng/mL and ≥180 ng/mL was 95.1 ± 5.4 days and 44.8 ± 14.3 days, respectively. Therefore, the mean duration of continued pregnancy was significantly longer for women with NE concentrations <180 ng/mL ($p = 0.047$).

Table 1. Clinical characteristics of women and neonates.

Maternal age (years)	30.9 ± 4.71 (22–40)
Gestational age at amniocenteses (weeks)	23.1 ± 1.76 (19–26)
Gestational age at cerclage (weeks)	23.4 ± 1.86 (19–26)
Nulliparous (number)	17
Parous (number)	20
Parity	1 (1–2)
With prior history of preterm birth (number)	9
Shirodkar cerclage (number)	29
McDonald cerclage (number)	5
Birthweight (g)	2169 ± 1017 (322–3640)
Apgar score at 5 minutes after birth	9 (0–9)[a]

Data are expressed as mean ± standard deviations (ranges) or numbers. Parity and Apgar score are expressed as median (range).
[a]There was only one case of stillbirth; the delivery took place at 19 weeks of gestational age after cerclage. There were no neonatal deaths.

Discussion

To our knowledge, this was the first study in which the association between levels of NE in amniotic fluid and the duration of continued pregnancy after therapeutic cervical cerclage was evaluated in pregnant women with bulging fetal membranes. Among women with amniotic fluid glucose levels ≥15 mg/dL and without intrauterine infections who underwent cervical cerclage, those with amniotic fluid NE levels <180 ng/mL were observed to continue their pregnancies for a significantly longer period compared with those with NE levels ≥180 ng/mL. At end-points of 30, 34, and 36 weeks of gestation, the positive predictive value of amniotic fluid NE levels for pregnancy continuation was 91.3, 91.5, and 80.9%, respectively, which indicate that sufficient continuation of pregnancy after cerclage can be expected in women with amniotic fluid NE levels <180 ng/mL before cerclage.

Several researchers have reported about the duration from cervical cerclage to delivery in pregnant women with bulging fetal membranes (Table 3) (3–5,20–29). In a previous study that did not involve the screening for intrauterine infection with preoperative amniocentesis, the postoperative duration of pregnancy was about 40–70 days, which was almost similar to our result of 44.8 ± 14.3 days for procedures performed only by amniotic fluid glucose ≥15 mg/dL. In contrast, Mays et al. (20) considered either amniotic fluid glucose <14 mg/dL or amniotic fluid lactate dehydrogenase ≥400 U/L to be signs of intrauterine infection. Cervical cerclage performed in pregnant women with neither amniotic fluid glucose <14 mg/dL or amniotic fluid lactate dehydrogenase ≥400 U/L led to a postoperative pregnancy duration of 93.4 ± 33.1 days, which was similar to the 95.1 ± 5.4 days observed in pregnant women who underwent the procedure at amniotic fluid ≥15 mg/dL and amniotic elastase <180 mg/dL in our study. Therefore, it may be necessary to combine several markers to screen for intrauterine infection.

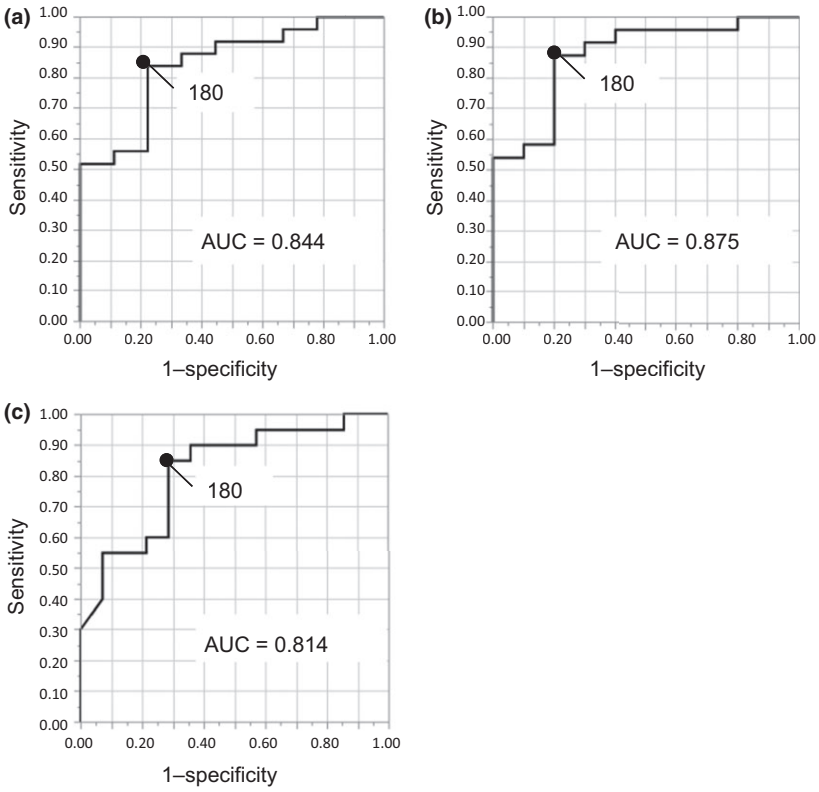

Figure 1. Receiver operating characteristics curves of amniotic fluid concentrations of neutrophil elastase (ng/mL) for predicting delivery beyond 30 (a), 34 (b), and 36 (c) weeks of gestation. AUC, area under the curve.

Table 2. Comparison of premature birth rate according to the use of combined amniotic fluid glucose and neutrophil elastase cut-off levels and the amniotic fluid glucose cut-off levels alone as an indication of cerclage.

Indication of cerclage	Delivery within 30 weeks of gestation	Delivery within 34 weeks of gestation	Delivery within 36 weeks of gestation
Glucose ≥15 mg/dL	26.5 (9/34)	29.4 (10/34)	41.1 (14/34)
Glucose ≥15 mg/dL and NE <180 ng/mL	13.6 (3/22)	13.6 (3/22)	27.2 (6/22)

Data are shown as % (numbers). NE, neutrophil elastase.

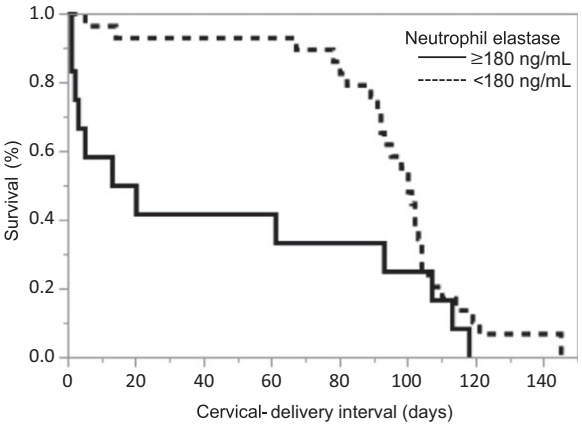

Figure 2. Kaplan–Meier curve of the duration from cervical cerclage to delivery (latency).

The utility of substances in the amniotic fluid for predicting the prognosis of cervical cerclage has only been described in a few studies. The use of IL-6 in amniotic fluid for predicting the prognosis of cervical cerclage has been reported in two studies (28,29). Diago Almela et al. (28) investigated whether IL-6 levels, white blood cell counts or glucose levels in amniotic fluid were more closely associated with pregnancy prognosis in 31 women with bulging fetal membranes. The authors observed that the amniotic IL-6 levels were most strongly correlated with the duration of continued pregnancy and premature birth rate, and that the associated cut-off value was 2.90 ng/mL. However, the limitations of that study include the possibility of selection bias due to the use of IL-6, white blood cell, and glucose levels to screen for intrauterine infection to determine whether cervical

Table 3. Selected descriptive data for cervical cerclage in women with bulging fetal membrane.

Reference	*n*	GA at cerclage (weeks)	Duration from cervical cerclage to delivery
Without evaluation of intrauterine infection			
Ochi, 1994 (21)	18[a]	16–25	35.2 ± 39.7 days
Althuisius, 2003 (22)	13	22.2 ± 3.3	54 ± 47 days
Daskalakis, 2006 (4)	29	18–26	8.8 weeks
Stupin, 2008 (23)	89	17–26	41 days
Schorr, 1996 (24)	42	22.5 (20.5–23.7)	30 (2–102) days
Ventolini, 2009 (25)	56	19.6 ± 3.2	9.1 weeks
Debby, 2007 (26)	24	16–27	9.3 ± 4.8 weeks
Khan, 2012 (3)	17	20.7 ± 4.0	11.65 ± 8.14 weeks
Nelson, 2009 (5)	18	21.3 ± 3.3	56.3 ± 46.4 days
Makino, 2004 (27)	17	22.9 ± 2.5	35.0 ± 41.3 days
With amniocentesis for evaluating intrauterine infection			
Mays, 2000 (20)	11	21.8 ± 1.5	93.4 ± 33.1 days
Present study	34[b]	22.2 ± 7.1	44.8 ± 14.3 (1–118) days
	22[c]	23.6 ± 6.0	95.1 ± 5.4 (13–147) days

Data are expressed as mean ± standard deviations (ranges). GA, gestational age.

[a]Amnioreduction before cerclage.

[b]Cerclage using only amniotic fluid glucose ≥15 mg/dL.

[c]Cerclage using both amniotic fluid glucose ≥15 mg/dL and neutrophil elastase <180 ng/mL.

cerclage is indicated. Furthermore, two women were included in the analysis despite the absence of intrauterine infection and not having undergone cervical cerclage because they did not consent to the procedure. Lee et al. (29) compared 40 women who underwent cervical cerclage for painless cervical dilatation of ≥2 cm (cervical insufficiency group) and 45 who did not exhibit cervical dilatation (control group). They reported that the optimal cut-off value for amniotic IL-6 level for predicting the continuation of pregnancy was 1700 pg/mL. Furthermore, they found that women with cervical insufficiency with IL-6 levels ≥1700 pg/mL had a significantly longer duration of pregnancy continuation compared with those with levels <1700 pg/mL (35 days vs. 2 days, respectively).

Interestingly, both the cut-off value for amniotic fluid NE level identified in the present study and the cut-off values for IL-6 levels reported in previous studies (1700–2900 pg/mL) were either equivalent to or lower than the mean values observed in chorioamnionitis-negative women, as determined in our previous study (9). This suggested that the presence of even mild inflammation may be sufficient to affect the subsequent continuation of pregnancy. In the present study, we only examined women considered to have a low likelihood of intrauterine infection based on amniotic glucose levels; however, some women exhibited high amniotic fluid NE levels, and a majority of these women underwent premature births. Therefore, it is necessary to use several markers to screen for intrauterine infections.

Neutrophil elastase has proteolytic activity and causes inflammation by itself. Both glucose and cytokines, such as IL-6, are useful markers of infection, but these have no ability to directly damage the tissue. Therefore, we believe that NE directly reflected the severity of infection. In our previous report, amniotic fluid NE was more sensitive than IL-6 for diagnosing amnionitis and funisitis (9). In this study, there were some women who had high NE levels regardless of whether they had glucose levels ≥15 mg/dL in the amniotic fluid. However, we cannot conclude that NE was a superior marker of infection based on our small sample size.

This study has some limitations. First, the sample size was small. Second, we performed cervical cerclage in all women without any intrauterine infection (based on amniotic fluid glucose levels ≥15 mg/dL) and did not include a control group. Therefore, we were unable to evaluate the effectiveness of therapeutic cervical cerclage itself. A prospective study should be conducted to investigate the durations of continued pregnancy in women without intrauterine infection who are randomly allocated to undergo or not undergo cervical cerclage.

In conclusion, the amniotic fluid NE level was found to be a useful clinical marker for predicting the continuation of pregnancy after cervical cerclage. Using NE as a marker of intrauterine infection can allow clinicians to estimate the effectiveness of therapeutic cerclage. We believe that the following prospective studies regarding the continuation of pregnancy after cervical cerclage will

be helpful: studies regarding amniotic fluid NE levels limited to only women with cervical shortening or bulging of the fetal membranes; comparative studies of NE and other inflammatory markers in amniotic fluid, such as IL-6; studies to determine surgical indications by assessing whether or not intrauterine infection is present based on NE levels alone; and studies including perinatal prognoses of infants.

Acknowledgment

We thank Mr. Nobuyuki Tabata, Diagnostic Business Department, Sanwa Kagaku Kenkyusho Co., Japan, for his generous help for the NE level measurements.

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