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Strip test for bedside detection of interleukin-6 in cervical secretions is predictive for impending preterm delivery

Abstract

Inflammatory cytokines in amniotic fluid are markers of prematurity which could characterize preterm labour of infectious origin. To avoid amniocentesis, we analyzed IL-6, IL-8, IL-10, and IL-13 by RT-PCR in cervical secretions (CS) of 307 women with preterm labour. IL-6 was detected in 26.3% patients who delivered at less than 34 weeks (specificity: 95.8%). In addition, IL-6 was associated with

delivery within 7 days (specificity: 91.6%). To render the detection more rapid and cheaper, a strip test was designed and evaluated comparatively with RT-PCR in 76 women. This bedside strip test was twice more sensitive than RT-PCR, with little decrease in specificity.

Full text

Despite advances in prenatal care, the rate of preterm delivery has remained unchanged over the past 20 years. Genital and intra-amniotic infections have been suspected as etiologic factors in 40-60% of spontaneous preterm deliveries [1]. CRP levels, WBC counts, and isolation of bacteria from cervical secretions (CS) and amniotic fluid are not sensitive enough to allow early diagnosis and treatment of those frequent subclinical infections. In contrast, the presence of proinflammatory cytokine IL-6 in amniotic fluid is associated with preterm delivery, short test-to-delivery intervals, neonatal morbidity, and has proven more sensitive and more specific than classical and molecular bacteriological tests [2, 3]. In order to avoid amniocentesis that can lead to complications, we analyzed cytokines in CS. Since ELISA assays in small samples of CS with high degrees of viscosity and turbidity are not easily reproducible, we analyzed IL-6, IL-8, IL-10, and IL-13 mRNAs by RT-PCR, and determined which cytokine correlated best with the outcome of pregnancy [4]. CS from 307 women hospitalized between 22 and 34 weeks with signs of preterm labour and with (n = 49) or without (n = 258) rupture of membranes, were collected by aspiration from the posterior fornix using a sterile plastic pipette after informed consent. Around 10^6 cells found per sample were enough to allow RNA extraction and RT-PCR. IL-6 was positive on the first day of hospitalization in 26.3% of the 95 women who delivered at less than 34 weeks (Table 1). Among the 212 women who delivered after 34 weeks, only 9 (4.2%) were positive ($p < 0.001$). The sensitivity of IL-6 mRNA for prematurity was 26.3%, the specificity was 95.8%. IL-6 was found in 33.3% of patients who delivered within 7 days and in 8.4% of patients who delivered more than 7 days later ($p < 0.001$). The sensitivity of the test for premature delivery within 7 days was 33.3%, the specificity 91.6%. The presence of IL-6 mRNA in CS from 10 patients without rupture of membranes, suggests either that IL-6 was produced within the cervicovaginal mucosa, or that subclinical chorioamniotitis was responsible for the migration of inflammatory cells. IL-8 was not associated with prematurity but with delivery within 7 days ($p < 0.005$). However its specificity (43.8%) was lower than that of IL-6 (91.6%). Neither IL-13 nor IL-10 were associated with prematurity or with

impending preterm delivery. Fibronectin (FDC-6 Fetal Fibronectin Immunoassay, CIS Bio International), a standard marker of preterm labour, was associated with prematurity and delivery within 7 days with a higher sensitivity (66.7% *versus* 26.3% for prematurity and 71.4% *versus* 33.3% for delivery within 7 days). This difference in sensitivity suggests that, whereas fibronectin correlates with prematurity of any etiology, IL-6 only reflects a risk of prematurity of infectious origin. In contrast, fibronectin has a lower specificity than IL-6 (80.0% *versus* 95.8% for prematurity and 70.8% *versus* 91.6% for delivery within 7 days).

To meet clinical requirements of rapidity, we designed a strip test assay at bedside for IL-6 in cervicovaginal secretions CS, similar to the urinary beta-HCG assay for pregnancy [5]. Immunochromatographic membranes (Pall Gelman Science, France) were coated with a mouse anti-human IL-6 mAb (clone B-E8, Diaclone, France) and a goat anti-human IgG Fc mAb (Diaclone) to make a test line and a control line, respectively. The sample of CS diluted 1:2 was applied onto a pad coated with a gold-labeled mouse anti-human IL-6 (clone B-E4, Diaclone) and affixed onto the membrane (Figure 1). In case of positivity, IL-6/gold-labeled anti-IL-6 antibody complexes bound to the test line. In the absence of IL-6, the free gold-labeled antibody bound to the control lane. The detection threshold of IL-6 was 20 pg/ml in CS in less than 15 min.

In order to evaluate this procedure, we tested 136 CS samples from 76 women hospitalized with signs of preterm labour and included according to the same criteria as in the first study. The samples were simultaneously tested by RT-PCR and immunochromatography (30 women had at least two determinations). IL-6 mRNAs were detected with the same frequency as in the first study and were still statistically associated with prematurity ($p < 0.001$) and preterm delivery within 7 days ($p < 0.001$). IL-6 protein was detected in a higher number of samples ($n = 28$) than IL-6 mRNAs ($n = 14$). The presence of IL-6 protein in the absence of IL-6 mRNAs could result from a contamination of the sample with amniotic fluid consecutive to membrane rupture. However, in 4 of those 14 patients no rupture was diagnosed, suggesting that the protein was produced by fetal membranes, decidua, or the fetus itself. In this second set of experiments, IL-6 strip test could detect both cervical and extra-cervical production of cytokine and was twice more sensitive than RT-PCR for the diagnosis of prematurity (30.8% *versus* 17.9%) and for the prediction of preterm delivery within 7 days (40.9% *versus* 22.7%). That increase in sensitivity was associated with little decrease in specificity for the diagnosis of prematurity

(93.8% *versus* 100%) or for impending preterm delivery (89.8% *versus* 95.9%) (Table 1).

CONCLUSION

This report describes for the first time the strip test detection of one cytokine in biologic samples. The presence of IL-6 in CS was predictive of impending preterm delivery. It was safe, easy, rapid, and cheap. It could become a method of choice to define a subset of patients with preterm labour of infectious origin, who could benefit of early antibiotherapy.

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Figures

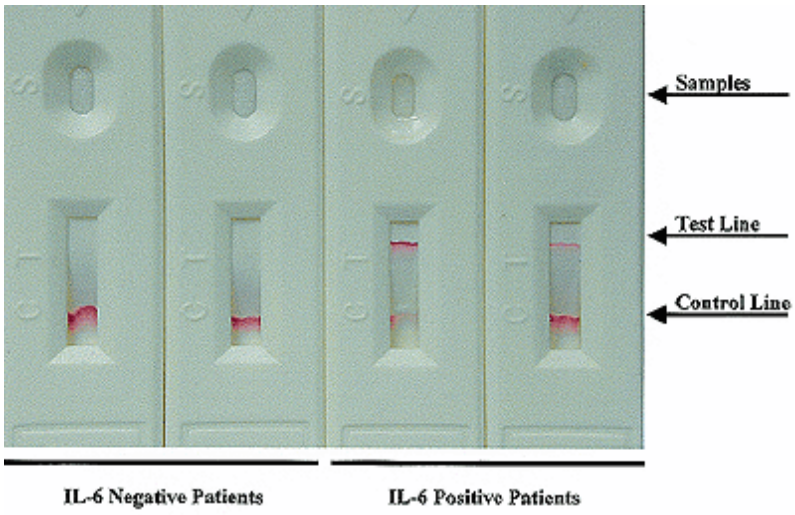


Figure 1

Strip test detection of IL-6 proteins in cervicovaginal fluids from women at risk for preterm delivery.

Table 1
Cytokine mRNAs and proteins in cervicovaginal fluids from women at risk for preterm labour
(* fibronectin was measured in only 268 patients)

	Prematurity			Test-to-delivery interval		
	≤ 34 wk n = 95 (78*)	> 34 wk n = 212 (190*)	p	≤ 7 days n = 33 (28*)	> 7 days n = 274 (240*)	p
Study 1						
IL-6 mRNA⁺ (n = 34)	26.3%	4.2%	0.001	33.3%	8.4%	0.001
IL-8 mRNA⁺ (n = 181)	64.2%	56.6%	N.S.	81.8%	56.2%	0.005
IL-10 mRNA⁺ (n = 189)	64.2%	60.4%	N.S.	72.7%	60.2%	N.S.
IL-13 mRNA⁺ (n = 56)	18.9%	17.9%	N.S.	21.2%	17.9%	N.S.
Fibronectin* > 50 U/l (n = 90)	66.7%	20.0%	0.001	71.4%	29.2%	0.001
Study 2	n = 78	n = 64		n = 44	n = 98	
IL-6 mRNA⁺ (n = 14)	17.9%	0.0%	0.001	22.7%	4.1%	0.001
IL-6 ICH⁺ (n = 28)	30.8%	6.2%	0.001	40.9%	10.2%	0.001

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