Clinical Policy Bulletin:  
Fetal Fibronectin, Inflammatory Biomarkers, and Salivary Estriol Testing for Preterm Labor  
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Policy  

Aetna considers the fetal fibronectin (fFN) immunoassay test medically necessary for evaluating symptomatic pregnant women at high-risk for preterm delivery (see background section for selection criteria). Repeat fFN immunoassay test is considered medically necessary if members remain symptomatic two or more weeks after a previous negative test.  

Aetna considers the fetal fibronectin test experimental and investigational for routine screening of the general obstetric population and for all other indications including high-risk women who are asymptomatic for preterm labor, for identifying optimal candidates for cerclage, and following insertion of a cervical cerclage because its effectiveness for these indications has not been established.  

Aetna considers biomarkers of intra-uterine inflammation (in amniotic fluid) including angiogenin, cytokines (e.g., interleukin-6, interleukin-8), maternal matrix metalloproteinase-9, and tumor necrosis factor-alpha experimental and investigational for evaluating pregnant women at high-risk for preterm delivery because their effectiveness for this indication has not been established.  

Aetna considers human chorionic gonadotrophin and phosphorylated insulin-like growth factor binding protein-1 (in cervico-vaginal fluid) experimental and investigational for evaluating pregnant women at high-risk for preterm delivery because their effectiveness for this indication has not been established.  

Aetna considers salivary estriol (SalEst) test experimental and investigational because the test results are not available rapidly enough to assist in decisions concerning the immediate care of the member.
Aetna considers ferritin, fructose bisphosphonate aldolase A, heat shock protein beta-1, peroxiredoxin-1, pyruvate kinase M1/M2, transferrin, uric acid and vimentin experimental and investigational as biomarkers for preterm labor because their effectiveness for this indication has not been established.

Aetna considers proteomic biomarkers (e.g., 14-3-3 protein sigma, annexin A5, inter-α-trypsin inhibitor heavy chain H4, protein S100-A8, and protein S100-A12) experimental and investigational for evaluating pregnant women at high-risk for preterm delivery because their effectiveness for this indication has not been established.

Aetna considers serum levels of angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), and the Ang-1/Ang-2 ratio levels experimental and investigational for evaluating pregnant women at high-risk for preterm delivery because their effectiveness for this indication has not been established.

**Background**

This policy is in accordance with the American College of Obstetricians and Gynecologists (ACOG) Committee Opinion on Fetal Fibronectin.

Fetal fibronectin (fFN) assay has recently been approved by the Food and Drug Administration (FDA) for clinical use in identifying patients at risk for preterm delivery. The fFN immunoassay is a qualitative test for the detection of fFN protein in cervico-vaginal secretions.

A number of studies have examined the value of the fFN test as a predictor of likelihood of preterm delivery in women with symptoms of preterm labor. The data indicate that a negative test has a maximal negative predictive value of approximately 96 % for not delivering within the next 2 weeks, while a positive test has a 15 to 20 % positive predictive value for preterm delivery. Despite these data, there have been no prospective interventional studies demonstrating a decrease in preterm deliveries or improved perinatal outcomes based on the knowledge of the results of this test. No study has examined the efficacy of fFN on the incidence, morbidity, and mortality of preterm delivery. However, there may be selected cases in which quickly available results may be helpful in assessing the patient's risk of preterm delivery allowing for an impact on clinical decisions.

In a Cochrane review on fFN testing for reducing the risk of preterm birth, Berghella and colleagues (2008) concluded that although fFN is commonly used in labor and delivery units to help in the management of women with symptoms of preterm labor, currently there is not sufficient evidence to recommend its use. Since this review found an association between knowledge of fFN results and a lower incidence of preterm birth before 37 weeks, further research should be encouraged.

Klebanoff and associates (2008) examined if salivary progesterone (P) or estriol (E3) concentration at 16 to 20 weeks' gestation predicts preterm birth or the response to 17alpha-hydroxyprogesterone caproate (17OHPC) and if 17OHPC treatment affected the trajectory of salivary P and E3 as pregnancy progressed.
This was a secondary analysis of a clinical trial of 17OHP to prevent preterm birth. Baseline saliva was assayed for P and E3. Weekly salivary samples were obtained from 40 women who received 17OHP and 40 who received placebo in a multi-center randomized study of 17OHP to prevent recurrent preterm delivery. Both low and high baseline saliva P and E3 were associated with a slightly increased risk of preterm birth. However, 17OHP prevented preterm birth comparably, regardless of baseline salivary concentrations of P and E3. Moreover, 17OHP did not alter the trajectory of salivary P over pregnancy, but it significantly blunted the rise in salivary E3 as well as the rise in the E3/P ratio. The authors concluded that 17OHP flattened the trajectory of E3 in the second half of pregnancy, suggesting that the drug influences the fetoplacental unit.

Selection Criteria for Fetal Fibronectin (fFN) Immunoassay:

According to the ACOG Committee on Obstetric Practice (1997), the fFN test is only appropriate for use in symptomatic pregnant women with all of the following characteristics:

- Amniotic membranes intact; and
- Cervical dilatation is minimal (less than 3 cm); and
- Sampling is performed no earlier than 24 weeks, 0 days and no later than 34 weeks, 6 days of gestation.

In addition, the fFN test is only useful if results are available quickly enough (generally considered to be under 4 hours) so that the results can assist in decisions concerning the immediate care of pregnant women.

Although a negative test appears to be useful in ruling out imminent preterm delivery (i.e., within 2 weeks), the clinical implications of a positive result have not been fully evaluated.

If the test is to be clinically useful, the results must be available from the laboratory in a timely manner (generally considered to be under 4 hours) so that the test can effect decisions concerning the immediate care of the patient. This is in accordance with the ACOG Committee Opinion, which states that "if the test is to be clinically useful, the results must be available from the laboratory in a timely manner."

ACOG does not recommend the fFN test for screening asymptomatic women to determine risk of preterm delivery. ACOG’s Division of Practice Activities concluded that “this test is not recommended as a routine screening procedure for the general prenatal population”. A recent clinical trial of the fFN test in 108 women at low risk of preterm delivery concluded that bi-weekly fFN determinations in asymptomatic women between 24 and 34 weeks’ gestation “are of limited clinical value for the prediction of preterm birth”.

Keeler and colleagues (2009) determined the relationship between fFN testing prior to ultrasound-indicated cerclage and obstetric outcome. Singleton pregnancies between 18 and 24 weeks’ gestation with an ultrasound-diagnosed short cervix (less than 25 mm) and funneling (greater than 25 %) of the chorio-amniotic membranes into the endocervical canal were analyzed. The fFN testing was performed and patients were randomized to cerclage or no-cerclage. Groups
were stratified by fFN result. Cerclage patients were compared with no-cerclage patients. The primary outcome was delivery prior to 35 weeks' gestation. Spontaneous preterm birth prior to 35 weeks' gestation occurred in 15 (44.1%) fFN-positive-cerclage patients and 16 (55.2%) fFN-positive no-cerclage patients ($p = 0.45$). Similarly, it occurred in 16 (17.8%) fFN-negative cerclage patients and 11 (17%) fFN-no-cerclage patients ($p = 0.99$). The authors concluded that fFN testing did not identify optimal candidates for cerclage.

Inflammatory biomarkers are being investigated as predictors of preterm birth. Gedc and Ford (2010) stated that there is overwhelming evidence that intra-uterine infection and inflammation play an important role in the pathogenesis of spontaneous preterm labor, preterm prelabor rupture of the membranes and fetal injury resulting in long-term sequelae. Early diagnosis of subclinical infection and inflammation may therefore aid clinicians institute interventions focusing on such adverse outcomes. Biomarkers of intra-uterine inflammation (e.g., interleukin-6) although sensitive, are not specific. Thus, decision to deliver remote from term because of intra-uterine infection and/or inflammation should be based on clinical signs and/or bacterial culture or Gram stain of amniotic fluid. In patients with preterm contractions and intact membranes, the risk of delivery is 1% within the week following a negative fFN in cervico-vaginal secretions. This aids to decide if antenatal steroids should be administered to patients presenting with preterm contractions between 24 and 34 weeks' gestation. Biomarkers in cervical secretions and amniotic fluid identify those who may benefit from cerclage when the cervix is shortened (less than 25 mm) and dilated in the second trimester. The authors concluded that so far, few interventions utilizing inflammatory biomarkers have shown clinical benefit. They noted that future efforts should focus on the quest for accurate biomarkers that can be obtained non-invasively and allow early prediction of subclinical disease to initiate appropriate risk-specific intervention.

In a case-control study nested in a large, prospective, multi-center cohort trial ($n = 5,337$), Kramer and colleagues (2010) examined the role of mid-trimester maternal plasma cytokines and C-reactive protein (CRP) as predictors of spontaneous preterm birth. Cohort women had an interview, examination, and venipuncture at 24 to 26 weeks. Frozen plasma samples in women with spontaneous preterm birth ($n = 207$) and approximately 2 term controls per case ($n = 444$) were analyzed using Luminex multi-analyte profiling technology. Fresh placental samples were fixed, stained, and blindly assessed for histological evidence of infection/inflammation, decidual vasculopathy, and infarction, and vaginal swabs were analyzed for bacterial vaginosis and fFN concentration. High maternal matrix metalloproteinase-9 (MMP-9) concentration, but none of the other cytokines or CRP, was significantly associated with spontaneous preterm birth [adjusted odds ratio $= 1.7$ (1.1 to 2.4)] and showed a dose-response relation across quartiles. No association was observed, however, between maternal MMP-9 and placental infection/inflammation, bacterial vaginosis, or vaginal fFN concentration. The authors concluded that these findings require confirmation in future studies, but suggest that a systemic immune response implicating MMP-9 may have an etiologic role in spontaneous preterm birth.

Conde-Agudelo et al (2011) examined the accuracy of novel biomarkers to predict spontaneous preterm birth in women with singleton pregnancies and no symptoms of preterm labor. Electronic searches in PubMed, Embase, Cinahl, Lilacs, and
Medion, references of retrieved articles, and conference proceedings were carried out. No language restrictions were applied. Observational studies that evaluated the accuracy of biomarkers proposed in the last decade to predict spontaneous preterm birth in asymptomatic women were selected. These researchers excluded studies in which biomarkers were evaluated in women with preterm labor. Two reviewers independently extracted data on study characteristics, quality, and accuracy. Data were arranged in 2 × 2 contingency tables and synthesised separately for spontaneous preterm birth before 32, 34, and 37 weeks of gestation. They used bi-variate meta-analysis to estimate pooled sensitivities and specificities, and calculated likelihood ratios (LRs). A total of 72 studies, including 89,786 women and evaluating 30 novel biomarkers, met the inclusion criteria. Only 3 biomarkers (proteome profile and prolactin in cervicovaginal fluid, and matrix metalloproteinase-8 in amniotic fluid) had positive LRs greater than 10. However, each of these biomarkers was evaluated in only 1 small study. Four biomarkers had a moderate predictive accuracy (interleukin-6 and angiogenin [a potent inducer of neovascularization], in amniotic fluid; human chorionic gonadotrophin and phosphorylated insulin-like growth factor binding protein-1, in cervico-vaginal fluid). The remaining biomarkers had low predictive accuracies. The authors concluded that none of the biomarkers evaluated in this review meet the criteria to be considered a clinically useful test to predict spontaneous preterm birth. They stated that further large, prospective cohort studies are needed to evaluate promising biomarkers such as a proteome profile in cervico-vaginal fluid.

The Royal College of Obstetricians and Gynaecologists’ clinical guideline on “Cervical cerclage” (RCOG, 2011) stated that fetal fibronectin testing following insertion of a cervical cerclage is not recommended.

Bamberg et al (2012) evaluated mid-trimester amniotic fluid concentrations of 3 major pro-inflammatory cytokines (interleukin 6 [IL-6], interleukin 8 [IL-8], and tumor necrosis factor-alpha [TNF-α]) in asymptomatic pregnancies with adverse outcomes. A prospective follow-up study at the Charite University Hospital, Berlin, Germany of women with uncomplicated singleton pregnancies at 2nd trimester and amniocentesis was carried out. Concentrations of IL-6, IL-8, and TNF-α were measured by enzyme-linked immunosorbent assay following amniotic fluid assessment by mid-trimester amniocentesis performed from gestation days 15 weeks 0 days up to 20 weeks 6 days. Values from normal pregnancies were compared to those from pregnancies having adverse outcomes of spontaneous abortion, preterm delivery, pre-eclampsia, or eclampsia. Main outcome measure IL-6, IL-8 and TNF-α in relation to adverse pregnancy outcome. A total of 298 consecutive patients were evaluated. Median patient age was 35 years (range of 19 to 43). Controls consisted of 273 women who delivered without further complications after 37 weeks gestation. The range values of IL-6, IL-8, and TNF-α in the control group were 4.9 to 2,620 pg/ml, 36.2 to 5,843 pg/ml, and 8.0 to 28.2 pg/ml, respectively. Patients with adverse pregnancy outcome (n = 25) were classified into 3 groups: (i) spontaneous abortion group (n = 4), (ii) preterm delivery group (n = 17), and (iii) pre-eclampsia/eclampsia group (n = 4). There were no significant differences in IL-6, IL-8, and TNF-α between controls and study groups, regardless of the type of complication (p > 0.05). The authors concluded that mid-trimester amniotic fluid concentrations of the pro-inflammatory cytokines IL-6, IL-8, and TNF-α are not predictive of adverse pregnancy outcome in terms of
spontaneous abortion, preterm delivery or pre-eclampsia/eclampsia in this study population.

Galazis and colleagues (2013) noted that preterm birth (PTB) is a major cause of neonatal mortality and morbidity. Women with polycystic ovary syndrome (PCOS) are at high-risk of PTB. There is a need for research studies to investigate the mechanisms linking PCOS and PTB, to facilitate screening, and develop novel preventative strategies. These researchers listed all the proteomic biomarkers of PTB and integrated this list with the PCOS biomarker database to identify commonly expressed biomarkers of the 2 conditions. They carried out a systematic review of PTB biomarkers and update of PCOS biomarker database. All eligible published studies on proteomic biomarkers for PTB and PCOS identified through various databases were evaluated. For the identification of the relevant studies, the following search terms were used: "proteomics", "proteomic", "preterm birth", "preterm labour", "proteomic biomarker" and "polycystic ovary syndrome". This search was restricted to humans only. A database on proteomic biomarkers for PTB was created while an already existing PCOS biomarker database was updated. The 2 databases were integrated and biomarkers that were co-expressed in both women with PCOS and PTB were identified and investigated. A panel of 6 proteomic biomarkers was similarly differentially expressed in women with PTB and women with PCOS compared to their respective controls (normal age-matched women in the case of PCOS studies and women with term pregnancy in the case of PTB studies). These biomarkers include pyruvate kinase M1/M2, vimentin, fructose bisphosphonate aldolase A, heat shock protein beta-1, peroxiredoxin-1 and transferrin. The authors concluded that these proteomic biomarkers (pyruvate kinase M1/M2, vimentin, fructose bisphosphonate aldolase A, heat shock protein beta-1, peroxiredoxin-1 and transferrin) can be potentially used to better understand the pathophysiological mechanisms linking PCOS and PTB. This would help to identify subgroups of women with PCOS at risk of PTB and hence the potential of developing preventative strategies.

Clowse et al (2013) stated that while increased disease activity is the best predictor of PTB in women with systemic lupus erythematosus (SLE), even women with low disease activity are at increased risk of this complication. Biomarkers that would identify at-risk pregnancies could allow interventions to prevent PTB. In this study, measures of SLE activity, inflammation, placental health and renal function between 20 and 28 weeks gestation (mid-gestation) were correlated to PTB and gestational age at delivery in a prospective cohort of pregnant women with SLE. Of the 40 pregnancies in 39 women, all with mild-moderate SLE disease, 9 (23.7%) of the 38 live births were delivered preterm. Low C4 was the only marker of SLE activity associated with younger gestational age at delivery. Elevated ferritin and lower estradiol correlated with younger gestational age at delivery. Renal function remained normal during all pregnancies at mid-gestation and did not correlate with PTB. Higher serum uric acid, however, correlated with younger gestational age at delivery. The authors concluded that in women with SLE with mild-moderate disease activity, ferritin, estradiol and uric acid levels at mid-gestation may predict PTB. They stated that these markers may prove to be clinically useful in identifying pregnancies at particularly high-risk for adverse outcomes.
Bastek and Elovitz (2013) stated that biomarkers associated with spontaneous PTB and pre-eclampsia have been discovered in patients who experience these adverse obstetrical outcomes. The identification of such biomarkers holds promise in both facilitating the early identification of those patients at greatest risk and enhancing the understanding of these disease processes to determine therapeutic interventions. These investigators reviewed the existing literature to determine the utility of biomarkers in the risk stratification of spontaneous PTB and pre-eclampsia. They found that despite the promise of some biomarkers in identifying patients at increased risk for spontaneous PTB and/or pre-eclampsia, the use of biomarkers in clinical practice to predict adverse obstetrical outcome remains challenging. Although data from small discovery studies may be encouraging, progress with biomarker research remains limited by the lack of validation of these discovered biomarkers. Furthermore, owing to the heterogeneity of existing studies, generalizable conclusions are difficult to understand, meta-analyses are challenging to perform, and agreement on cut-point standardization is difficult. The authors concluded that the identification of an abnormal biomarker level does not guarantee whether or when an adverse clinical event might occur. The lack of understanding of the true etiologies of these disease processes resulting in the absence of definitive interventions to prevent spontaneous PTB and pre-eclampsia from occurring.

Schneuer et al (2014) evaluated angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), and the Ang-1/Ang-2 ratio levels in the first trimester of pregnancy, their association with adverse pregnancy outcomes, and their predictive accuracy. This cohort study measured serum Ang-1 and Ang-2 levels in 4,785 women with singleton pregnancies attending first trimester screening in New South Wales, Australia. Multi-variate logistic regression models were used to assess the association and predictive accuracy of serum biomarkers with subsequent adverse pregnancy outcomes (small for gestational age, PTB, pre-eclampsia, miscarriage greater than 10 weeks, and stillbirth). Median (interquartile range) levels for Ang-1, Ang-2, and the Ang-1/Ang-2 ratio for the total population were 19.6 ng/ml (13.6 to 26.4), 15.5 ng/ml (10.3 to 22.7), and 1.21 (0.83 to 1.73), respectively. Maternal age, weight, country of birth, and socioeconomic status significantly affected Ang-1, Ang-2, and the Ang-1/Ang-2 ratio levels. After adjusting for maternal and clinical risk factors, women with low Ang-2 levels (less than 10th percentile) and high Ang-1/Ang-2 ratio (greater than 90th percentile) had increased risk of developing most adverse pregnancy outcomes. Compared with the Ang-1/Ang-2 ratio alone, maternal and clinical risk factors had better predictive accuracy for most adverse pregnancy outcomes. The exception was miscarriage (Ang-1/Ang-2 ratio area under receiver operating characteristic curve = 0.70; maternal risk factors = 0.58). Overall, adding the Ang-1/Ang-2 ratio to maternal risk factors did not improve the ability of the models to predict adverse pregnancy outcomes. The authors concluded that these findings suggested that the Ang-1/Ang-2 ratio in first trimester is associated with most adverse pregnancy outcomes, but do not predict outcomes any better than clinical and maternal risk factor information.

Kacerovsky et al (2014) analyzed the findings of studies on proteomic biomarkers for spontaneous PTB. Three electronic databases (Medline, Embase, and Scopus) were searched for studies in any language reporting the use of proteomic biomarkers for PTB published between January 1994 and December 2012.
Retrieved citations were screened, and relevant studies were selected for full-text reading, in triplicate. The search yielded 529 citations, 51 were selected for full-text reading and 8 studies were included in the review. A total of 64 dysregulated proteins were reported. Only 14-3-3 protein sigma, annexin A5, protein S100-A8, protein S100-A12, and inter-α-trypsin inhibitor heavy chain H4 were reported in more than 1 study, but results could not be combined due to heterogeneity in type of sample and analytical platform. The authors concluded that according to the existing literature, there are no specific proteomic biomarkers capable of accurately predicting PTB.

CPT Codes / HCPCS Codes / ICD-9 Codes

CPT codes covered if selection criteria are met:

82731 Fetal fibronectin, cervicovaginal secretions, semi-quantitative quantitative [not covered following insertion of a cervical cerclage]

CPT codes not covered for indications listed in the CPB:

82085 Aldolase [fructose bisphosphonate aldolase A]
82677 Estriol
82728 Ferritin
83516 Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; qualitative or semiquantitative, multiple step method
83518 qualitative or semiquantitative, single step method
83519 qualitative by radioimmunoassay (e.g., RIA)
83520 qualitative, not otherwise specified
84210 Pyruvate [pyruvate kinase M1/M2]
84466 Transferrin
84550 Uric acid; blood
88324 Immunohistochemistry or immunocytochemistry, each separately identifiable antibody per block, cytologic preparation, or hematologic smear

Other CPT codes related to this CPB:

59320 Cerclage of cervix, during pregnancy; vaginal

HCPCS codes not covered if selection criteria are met:

S3652 Saliva test, hormone level; to assess preterm labor risk
ICD-9 codes covered if selection criteria are met [not covered for biomarkers of intra-uterine inflammation such as cytokines or maternal matrix metalloproteinase-9]:

622.5 Incompetence of cervix
644.00 - 644.21 Early or threatened labor
654.50 - 654.54 Cervical incompetence during pregnancy, childbirth, and the puerperium
V23.41 Supervision of pregnancy with history of pre-term labor

Other ICD-9 codes related to the CPB:

646.30 - 646.33 Habitual aborter
V22.0 - V22.1 Supervision of normal pregnancy
V23.49 Supervision of pregnancy with other poor obstetric history
V23.5 Supervision of pregnancy with other poor reproductive history
V28.8 - V28.9 Antenatal screening

The above policy is based on the following references:


35. Mundy L, Merlin T, Parrella A. A rapid foetal fibronectin assay as a predictive test for women suspected of being in pre-term labour. Horizon Scanning Prioritising Summary - Volume 6. Adelaide, SA: Adelaide Health Technology Assessment (AHTA) on behalf of National Horizon Scanning Unit (HealthPACT and MSAC); 2004;6.


37. Medical Services Advisory Committee (MSAC). Fetal fibronectin test for preterm labour. MSAC Application 1103. Canberra, ACT: Medical Services Advisory Committee (MSAC); 2006.


