Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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TITLE: Prediction of Preterm Birth

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Slide 1: Title slide

Slide 2:
Preterm birth (PTB) is defined by the American College of Obstetricians and Gynecologists (ACOG) as delivery of an infant prior to 37 weeks gestation. There are two main types of PTB: spontaneous and induced. Approximately 20% of all PTBs are induced. In most cases, induction is medically necessary due to danger to the mom or fetus. 80% percent of PTBs are spontaneous with and without premature rupture of membranes. This talk will focus on spontaneous PTB. PTB accounted for ~12.7% of live births in 2007 and this incidence has risen by >20% since 1990. This increase continues despite advances in prediction and prevention of PTB. Premature delivery of an infant accounts for >75% of fetal and neonatal deaths in babies without genetic anomalies, and infants who survive a premature birth suffer significant long term morbidity. The morbidities are most commonly secondary effects of respiratory distress syndrome which occurs when fetal lungs fail to mature. These include: bronchopulmonary dysplasia, intraventricular hemorrhage, neurodevelopmental problems and cognitive difficulties.

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While numerous studies have examined potential preventative measures for PTB, few have been successful. Traditional treatments include hydration, bed rest, and home uterine activity monitoring, and these have a low success rate. Tocolytic therapy, aimed at stopping contractions, has been used with limited success. The latest studies suggest that it can be used only to PROLONG pregnancy for a short period of time, but not to prevent birth. A recent meta-analysis showed that 17-hydroxyprogesterone, when given beginning at 16 weeks gestation to women with a history of PTB, is effective in preventing PTB. There is insufficient evidence to support its use in any other setting. Therefore, in most women, the goal of therapy is to prolong pregnancy long enough to administer antenatal corticosteroids. Injection of dexamethasone or other corticosteroids at least 48 hours prior to delivery of an infant induces lung maturity and significantly reduces morbidity and mortality associated with PTB. Thus, it is crucial that we identify an accurate predictor of PTB. One reason that it has been so challenging to find appropriate therapy for patients with PTB is that this is a diverse syndrome with numerous etiologies.

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Extensive literature review supports the idea that the majority of PTBs occur due to one (or more) of four major pathobiological processes. Each of these pathways culminates into a final common pathway that involves induction of uterine contractions and cervical changes with or without premature rupture of membranes.

These pathways include:
1) Induction of the maternal or fetal stress response system through activation of mom and/or baby's hypothalamic-pituitary-adrenal axes. Stress accounts for ~15% of PTBs.
2) Inflammation of the chorion, amnion, or decidual areas and/or systemic inflammation can lead to elevation of cytokines which act on the chorionic and/or decidual surfaces to induce labor. Inflammation with or without infection accounts for the majority of PTBs (up to 50%).
3) Decidual hemorrhage leading to detachment of the placenta (commonly called placental abruption) accounts for ~10% of PTBs, and in many cases is due to a bleeding or coagulation disorder.
4) The fourth known etiology of PTB is pathobiological distention of the uterus due to polyhydramnios (too much amniotic fluid) and/or multiple gestation.

These may occur simultaneously or separately. Each is a distinct pathobiological process that may respond differently to therapy. Since each etiology is different, prediction of PTB in women with overlapping symptoms may be very challenging.

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Clinical signs and symptoms of labor including contractions, abdominal pain, and cervical dilation occur late in the process and are very non-specific. In fact, the majority of women with labor symptoms will not deliver Preterm. In women with true preterm labor, symptoms often occur too late to intervene. Therefore, there is a real need for a biochemical marker that can predict PTB early in the process.

Genetics are a good predictor or PTB. A mother born prematurely (less than 30 weeks gestation) has a 2.4X increased risk of delivering her child preterm. Further, there is a huge disparity among races. African-Americans are ~twice as likely as Caucasians to deliver preterm when other etiological and socioeconomic factors are well controlled (we're going to talk more about this later).

One of the best predictors of PTB among multiparous pregnant women is a history of PTB. One prior PTB incurs a 15% increased risk, while 2 prior PTBs predispose a mother to a 32% increased risk of delivering a subsequent child preterm. This population has a therapy, 17-Hydroxyprogesterone, that works, and is recommended for use by the ACOG. The problem is that primary predictors based on risk factors like history, socioeconomic status, lifestyle, etc… provide ~20% sensitivity and a Positive Predictive Value (PPV) of 30% so alone they are not good predictors of PTB. This is partially due to the different etiologies of disease, and partially due to the fact that half of all PTB occurs in women with no known risk factors.

Cervical evaluation by digital exam is also not reliable, but there are different data indicating that measuring cervical length by transvaginal ultrasound is a good predictor of PTB, especially when combined with other biochemical markers.

Several biomarkers and genetic factors have been tested for clinical use in predicting PTB including salivary estriol and cytokines, but only fetal fibronectin (fFN) is recommended by the ACOG for routine prediction of PTB.

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fFN is an extracellular matrix (ECM) glycoprotein with significant homology to fibronectin. During pregnancy, its expression in the ECM at the junction between the maternal decidua and the fetal chorionic membranes may be important for implantation and maintenance of fetal/maternal attachment. Immunoassays specific for the unique oncofetal domain of fFN can detect fFN in cervicovaginal secretions of women for the first 22 weeks of their pregnancy until the fetal membranes completely fuse to the maternal decidua. By 37 weeks gestation, fFN becomes more heavily glycosylated, loses its adhesive properties and can again be detected in cervicovaginal secretions. fFN is thought to be present in the cervicovaginal fluid during spontaneous preterm labor (PTL) as a result of a mechanical separation of the chorion from the decidual membrane, or sloughing/secretion in response to chronic local inflammation. The clinical utility of fFN is in its high Negative Predictive Value (NPV). Specifically, a negative test is a good predictor of women who will not deliver within 14 days of testing.

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As mentioned on the previous slide, fFN is detectable in cervicovaginal secretions during the first 22 weeks of pregnancy. Its expression pattern by gestational week is depicted here. Typically, fFN expression is undetectable in cervicovaginal fluid (CVF) between gestational weeks 24 and 35. Numerous studies have reported that a CVF fFN concentration greater than 50 ng/mL between 24 – 34weeks 6days gestation is associated with an increased risk for preterm delivery in both symptomatic and asymptomatic women.

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In the laboratory, fFN is measured using a rapid semi-quantitative assay or a manual enzyme immunoassay. Both assays use a proprietary antibody manufactured by Hologic and the test is FDA approved in the following settings:

In women with symptoms of PTL who are between 24 – 34weeks 6days gestation, fFN measurements can detect with reasonable certainty women who will not delivery within 7 or 14 days of testing. The high NPV (>99%) allows physicians to send patients home instead of admitting them to the hospital. The clinical utility of fFN in asymptomatic women is less clear. The manufacturer states that in women with a history or other clinical suspicion for PTB, measurement of fFN every 2 weeks helps to assess a woman's risk of delivering prior to 35 weeks gestation. The NPV in this population is ~96%. In each case, the PPV is low (~15%). Clinicians have a difficult time deciding how to act on a positive fFN test result. fFN is also contraindicated in several situations, many of which occur in women with PTL, including advanced cervical dilation, premature rupture of amniotic membranes, in women with cervical cerclage, vaginal bleeding, or a history of sexual intercourse within 24 hours of testing.

The problem with fFN testing is that even in symptomatic women, only about 5% will actually deliver preterm. Any diagnostic marker would demonstrate a similar NPV because the prevalence of disease is so low! The NPV of a coin flip in a disease with a 5% prevalence is 95%. What we actually need is a biomarker with a high PPV. Because of its presence in inflammatory disease, we and others sought to investigate whether Interleukin -6 (IL-6) measured in CVF would be a better predictor of women who would deliver preterm.

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50% of PTBs are associated with infection or inflammation. If left untreated, infections of the amniotic cavity can lead to chorioamnionitis (CAM). This is a serious infection which is rarely identified in pregnancy because few women have clinical symptoms (only about 12.5%). Most often, CAM is diagnosed through a pathological evaluation of the placenta. In patients with CAM, there is a 4x increased risk of neonatal mortality. This is in part due to the increased risk of PTB. CAM triggers expression of proinflammatory cytokines which in turn signal the release of prostaglandins to induce cervical ripening and uterine contractions. Because of their presence during CAM and their role in the pathobiology of disease, cytokines measured in both cervicovaginal and amniotic fluid have been investigated as potential biomarkers for the prediction of PTB. Among them, CVF IL-6 has shown the most promise. How does this compare to fFN testing?

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We recently performed a large multicenter trial looking at the diagnostic utility of fFN and IL-6 measured in CVF to predict PTB within 14 days of fFN testing. The study utilized 725 samples from 660 women with and without symptoms of labor. Positive results for both markers were significantly associated with delivery of an infant within 14 days of fFN testing. Interestingly, IL-6 performed similarly to fFN in that both had a high NPV, but the PPV was low. Likelihood ratios (LR) are actually a better estimate of the diagnostic accuracy of a biomarker. In this case, the LR+ depicts how likely a positive test result is associated with PTB, while LR- describes how likely a negative test result is associated with a full term birth. A test with strong diagnostic accuracy in a rule out situation has a LR- of <0.1, while LR+ is >10 in a test with strong ability to rule in disease. As you can see, neither fFN nor IL-6 are strong diagnostic markers to rule in or out PTB. Why are we unable to find a good diagnostic marker for PTB? One thought is that this is a complex syndrome with different underlying etiologies. This argument is supported in recent literature looking at racial disparities in PTB.

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As I mentioned earlier, the overall prevalence of PTB has increased by 20% in the last 20 years. Interestingly, when broken up by race, this is not a global phenomenon. Since 1990, a significant increase (~35%) has been observed in the Caucasian population. This increase is attributed to increases in rates of assisted reproductive technology and multiple gestations. Surprisingly the prevalence has remained largely unchanged among African-Americans. Why is the rate of PTB so much higher in African-Americans and why the continued disparity? Some suggest differences in socioeconomic status, time at presentation for obstetrics care, and/or prevalence of early teen pregnancies. However, studies show that disparities in PTB rates still exist when socio-economic status and other risk factors are normalized, suggesting an underlying genetic difference that predisposes African-American women to increased risk of PTB.

Preliminary studies on the topic have demonstrated differential protein expression for some cytokines including IL-6 in amniotic fluid of women who delivered preterm. These differences in protein expression correspond with differences in single nucleotide polymorphisms present in cytokine genes between African-American and Caucasian women.

Do these differences translate to differences in diagnostic utility of fFN and IL-6 among different races?

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In a large multicenter study in which patients were divided by race, fFN in the Caucasian and IL-6 in the African-American population showed improved diagnostic strength with LR+ >5 being a test with moderate rule in ability. This finding makes sense when we consider recent literature demonstrating a genetic difference between African-American and Caucasian women who deliver preterm based upon large scale single-nucleotide polymorphism (SNP) analysis.

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These studies were the first to suggest a genetic predisposition to etiology of PTB. In the population studied, PTB in African-American women was most often due to infection/inflammation, while PTB in Caucasian women was due to hematologic or cervical disease as well as inflammation. Currently, experts in the field believe that PTB results from complex interactions between genes and environment. Therefore, the best predictor of PTB may be one that accounts for race and etiology.

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Nationally, we are faced with the epidemic of increasing PTB rates despite significant research into prediction and prevention. Part of the problem is that we don't have a complete understanding of the underlying pathophysiologies leading to preterm labor and birth. Further, we continue to struggle with prediction of which pregnant women with and without symptoms of PTL will deliver preterm. The presence of IL-6 or fFN in CVF may indicate an increased risk for preterm delivery, however these markers are non-specific. The absence of IL-6 or fFN in the CVF of women with and without symptoms of labor is an excellent predictor of women who will not deliver preterm with a NVP >98%, but in a low prevalence disease like PTB this is not much better than a coin flip. Recent evidence suggests that genetic differences between African-American and Caucasian women who deliver preterm lead to completely different pathobiological processes of disease. Prediction of PTB may require a different kind of analysis- one looking at the interaction of multiple markers secreted in different pathobiological processes by patients of different racial/ethnic backgrounds.

Slide 15: References