Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
www.traineecouncil.org

TITLE: Fetal Lung Maturity Testing

PRESENTER: Ann M. Gronowski, PhD

Slide 1: Title Slide

Slide 2:
Before I talk about laboratory testing for fetal lung maturity (FLM), I would like to describe the cause of respiratory distress syndrome (RDS). Then I would like to describe the different laboratory tests we have available for measuring FLM and the clinical utility of these tests. Finally, I would like to review recent studies that demonstrate minimal impact of FLM testing on clinical outcomes.

Slide 3:
Respiratory distress that occurs within the first few hours of life is referred to as infant RDS or hyaline membrane disease. It is most commonly caused by a deficiency in pulmonary surfactant due to premature birth. The pulmonary surfactants are needed to help reduce the surface tension in the alveoli of the infant lung and prevent the alveoli from collapsing. There are other more rare causes of RDS including genetic defects that cause altered production of surfactant proteins. RDS is the most common cause of respiratory failure in neonates and occurs with increasing frequency with decreased gestational age such that it is very common in infants born before 28 weeks and very rare in term infants. It's estimated that approximately 20,000 infants develop RDS each year in the United States. The best way to avoid RDS is to prevent premature birth.

Slide 4:
This slide shows the major phospholipid surfactants involved in FLM. These phospholipids are phosphatidlycholine, phosphatidylglycerol, phosphotidylinositol, and sphingomyelin. Over 50 years ago, it was demonstrated that measuring the phospholipids in amniotic fluid allowed accurate prediction of FLM.

Phosphatidlycholine, also known as Lecithin, accounts for approximately 80% of the phospholipids present in the surfactant. Lecithin is a critical component of surfactant because it contributes the most to lowering the surface tension of the alveoli. Also, lecithin increases in concentration from approximately 28 weeks of gestation through birth, with peak production occurring at 36 weeks of gestation. Sphingomyelin accounts for approximately 2% of the phospholipids present in the surfactant.

Lecithin is expressed as the lecithin to sphingomyelin ratio (L/S ratio) in order to account for any amniotic fluid (AF) volume changes, because sphingomyelin remains largely constant in the third trimester of gestation and serves as an internal standard in the L/S ratio. Phosphatidylglycerol, commonly abbreviated as PG, accounts for approximately 5-15% of the phospholipids present in the surfactant. PG appears in the fetal lung at 36 weeks gestation and continues to increase until term.

Slide 5:

As I mentioned already, predicting the risk that an infant will have RDS if delivered within 72 hours of when testing is performed can be done by assessing the surfactants present in AF. This testing is really only useful between 32 and 39 weeks of gestation. Prior to 32 weeks, the infant stands the risk of other morbidities associated with pre-term birth such as intracranial hemorrhage, inability to eat, or maintain body temperature, etc. FLM testing should only be performed if delivery is desired, but could be safely delayed. The American College of Obstetricians and Gynecologists (ACOG) guidelines do not recommend FLM testing if delivery is mandated for fetal or maternal indications. If delivery is imminent or if delivery must occur in order to prevent morbidity or mortality in the mother, then there is no point in FLM testing. These tests allow physicians to make decisions about delivery by weighing the risk to mom of not delivering vs the risk to baby of delivering. As a screening tool, these tests must have high sensitivity to detect RDS so that infants are not inappropriately delivered only to develop respiratory distress.

Slide 6:

This slide illustrates the most commonly used laboratory tests for assessing FLM and their clinical utility. We will briefly discuss each of these tests separately. The L/S Ratio was the first biochemical test for assessing FLM. For many years, it was considered the gold standard. However, you can see that it actually has lower clinical sensitivity than more modern methods such as lamellar body count (LBC).

Slide 7:

The L/S ratio is a thin layer chromatography (TLC) test that is quantified by densitometry. The L/S ratio describes the relative change in the concentration of lecithin to that of sphingomyelin in the AF. The greater the L/S ratio, the more mature the fetal lungs are. The risk of respiratory distress is exceedingly low when the L/S ratio is greater than 2.0 to 2.5. It is important to note that this test is the most difficult to perform because it requires considerable skill and expertise. Additionally, this test takes several hours to perform. Testing results are also affected by blood and meconium when they are present in the AF.

Slide 8:

The presence or absence of PG was not originally a part of the L/S ratio. However, it was added in order to decrease the number of false immature results. By adding PG to the L/S ratio when you do TLC, the sensitivity of the L/S ratio was maintained while increasing test specificity. A mature result is PG >2% of total phospholipid. The main advantage of measuring PG is that it is not affected by blood or meconium. However, if the PG is performed by TLC and expressed as a ratio of PG/S, the test is not valid in the presence of blood, since sphingomyelin is present in blood. PG has the disadvantage of being the last lipid to increase in surfactant (around gestational week 36). Therefore, a mature result is good evidence that the infant will not develop RDS.

Slide 9:
A commercially available agglutination test for the qualitative detection of PG is available. PG from the AF is incorporated into lipid particles. Anti-PG antibodies are then added. If PG is present in the AF, then there is agglutination of the lipid particles. Visible inspection of the agglutination reaction is conducted to determine the presence or absence of PG. The results from this test are qualitative and reported as either "negative", "low" positive, or "high" positive. Testing results are not affected by blood and meconium when they are present in the AF.

Slide 10:
The surfactant to albumin ratio can be measured using fluorescence polarization. A fluorophore is added to the AF. It can attach to albumin, which slows its rotation. It moves more rapidly when it is associated with the phospholipids. The change in polarization, which is a function of how rapidly a fluorophore is rotating, depends on the microviscosity of the solution. An elevated surfactant/albumin ratio has been correlated with the presence of FLM; the threshold for maturity is 55 mg of surfactant per gram albumin. Test results are affected by blood and meconium when they are present in the AF. This was a commercially available kit. However, production of this kit has been discontinued.

Slide 11:
Lamellar bodies are packages of phospholipids that are made by the type 2 pneumocytes in lung alveoli. The most recent FLM test was developed in the mid-1980s when it was noted that lamellar bodies were similar in size to blood platelets and could be enumerated using an automated cell counter. Lamellar Body Count testing is a laboratory developed test. A hematology analyzer is used to analyze the AF in the CBC mode and the lamellar bodies are counted as platelets. The more lamellar bodies present, the more mature the fetal lungs are. Lamellar body counts of 50,000 per microliter or greater suggest pulmonary maturity. However, results are instrument-dependent and need to be validated in individual laboratories. Test results are affected by blood and meconium when they are present in the AF.

In summary, all of these laboratory tests can be used for assessing FLM. They all have their advantages and disadvantages. However, the one thing they all have in common is that they are excellent predictors of FLM but not of fetal lung immaturity.

Slide 12:
Interestingly, despite the excellent sensitivity of these tests for predicting infants who will develop RDS, testing is done almost exclusively in the US. However, mortality due to RDS is greater in the US than the UK or Canada! Furthermore, several studies confirm a decrease in the volume of FLM testing done in the US over the last 10 years.

Slide 13:
Why is FLM testing decreasing in the US? One likely answer is a decrease in elective delivery. An interesting study from 2009 by Tita et al examined 13, 258 elective C-sections between 37 and 39 weeks of gestation. They found that infants born at 37 or 38 weeks, as compared to infants born at 39 weeks,

had increased poor outcomes, including adverse respiratory outcomes, the need for mechanical vent, newborn sepsis, hypoglycemia, admissions to the NICU, and hospitalization >5d. These findings support recommendations to delay elective delivery until at least 39 weeks of gestation.

Slide 14

As an interesting follow-up to that 2009 study, the same group compared the two groups of infants that we would think are "OK" to deliver. They compared 459 infants between 36-38 weeks of gestation with a mature FLM test result vs 13,339 infants 39-40 weeks of gestation. They found that neonates delivered at 36-38 weeks after confirmed FLM are still at higher risk of adverse outcomes than those delivered at 39-40 weeks. They recommend that purely elective FLM testing and early delivery should be avoided.

Slide 15:

In summary, I hope you have a better understanding of the most common cause of infant RDS and increased knowledge of the different laboratory tests we have available for measuring FLM and their clinical utility. Finally, you should have some understanding of why the demand for FLM testing is decreasing.

Slide 16:

I would like to acknowledge and thank Dr. David Grenache. Also, thank you for your time today. I hope you found this presentation on Fetal Lung Maturity informative and helpful.

Slide 17: References