Assessment of Fetal Lung Maturity: in Search of the Holy Grail

In this issue, Chapman et al. (pp 1974–7) evaluate a commercial enzymatic assay for phosphatidylglycerol (PG). They have adapted the method for use on a centrifugal analyzer, and demonstrate that the results of this approach compare favorably with other assessments of PG, but are obtained more rapidly and cost-effectively.

This work reminds us that, despite the passing of nearly two decades since the pioneering work of Gluck et al. (1), the clinical and laboratory communities are still in pursuit of a reliable method for assessing fetal lung maturity (FLM). The prediction of respiratory distress syndrome (RDS) presents us with a formidable problem: a disease with an overall low prevalence, and a test [amniotic fluid phospholipids by thin-layer chromatography (TLC)] that requires considerable laboratory time, expense, and expertise. Because the issue concerns the lives of infants who might develop a life-threatening condition (RDS) if delivered pre-term (even if other concerns suggest the desirability of early delivery), a strong emotional component overlies the debate of what should be done to test for this disorder in the laboratory.

Of greatest concern in assessing FLM is the detection of every case that will develop RDS. That is, we demand very high sensitivity, to afford a high predictive value of a negative ("mature") test result for the nonoccurrence of RDS. In return, we pay the price of relatively poor specificity, with many positive ("immature") results being falsely positive. For newborns of 37 weeks or greater gestation, there is a <1% chance that the infant will develop RDS. Thus, in this group, a test with even 99% specificity (higher than any test currently or likely to be available to assess FLM) will have a predictive value of a positive result of ≤50%; the false-positive results will be comparable with or greater than the true positive results even for such a relatively specific test. On the other hand, the probability of RDS increases rapidly with decreasing gestational ages—from about 30% at 34 weeks to >60% at 29 weeks. For these infants, reasonable predictive values of an immature result are found with tests of much lower specificity. Thus, we are confronted with the fact that the predictive value for RDS of an immature result is high only in those situations (<34 weeks of gestational age) in which one can virtually take for granted, based solely on fetal age, that RDS is likely. For the group of infants with which we are most often confronted with therapeutic decisions (34–37 weeks), the predictive value of an immature result is low, and much lower yet for near-term infants.

Originally, only the lecithin/sphingomyelin ratio (L/S) was determined in the TLC procedure (1), with L/S ≥ 2.0 generally being regarded as the criterion for a prediction of maturity. Subsequently, researchers learned that this approach could lead to unacceptable false predictions of maturity in certain situations, particularly among diabetic mothers. Adding PG to the TLC determination largely eliminated such false negatives (2). In fact, most workers today believe that the presence of PG in amniotic fluid nearly precludes RDS. The determination of PG is additionally attractive because, of all approaches used today, measurement of PG is least affected by contamination of the amniotic fluid with other substances (e.g., blood or meconium); we may use this excellent test in nearly every setting. However, the specificity of PG remains relatively low (about 70%), with a resulting predictive value of an immature result being typically about 30%. Most clinicians would be willing to accept these limitations. Unfortunately, the clinical laboratory cannot generally provide this service by TLC 24 h a day, seven days per week, because of the labor and skill level required.

We have thus continued to pursue the Holy Grail by seeking faster, easier, and cheaper methods that would offer the same information. For example, the community has now had several years' experience with a latex agglutination method that has proved promising for detecting PG. However, some laboratorians are uncomfortable with reading such a slide test because they feel it is somewhat subjective, most obviously so at low concentrations of PG. Further, many workers initially felt this test did not possess sufficient analytical sensitivity (3), although the vendor has subsequently improved the product, as noted in this issue by Chapman et al. and by others previously. Further, the reagents for this approach are not inexpensive. An enzymatic approach, on the other hand, could provide the desired combination of simplicity and analytical sensitivity, as well as lower cost, especially when partially automated, as presented by Chapman et al. This approach thus warrants continued evaluation and consideration.

Many other methods for the prediction of FLM have been introduced over the years. Their greatest virtues have been speed, low cost, and simplicity; nonetheless, they have not been broadly accepted in lieu of TLC determinations of L/S and (or) PG. Such approaches generally rely on a biophysical characteristic of amniotic fluid rather than its chemical constituents. Examples include the shake test (4), which has been subsequently improved and made suitable for commercial production (5). In this approach, the amniotic fluid is mixed with various concentrations of ethanol, shaken, and observed for a stable ring of foam. Like latex agglutination for PG, the reading of this method is considered somewhat subjective by many, especially for borderline cases.

Other biophysical approaches that have been in the literature for many years include macroscopic surface tension (6) and assessment of the fluidity of surfactant micelles, often called amniotic fluid "microviscosity," by fluorescence depolarization (7). Surface tension methods are technically difficult and rarely used; the microviscosity

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1 Nonstandard abbreviations: PG, phosphatidylglycerol; FLM, fetal lung maturity; RDS, respiratory distress syndrome; TLC, thin-layer chromatography; and L/S, lecithin/sphingomyelin ratio.
approach on the other hand, has experienced renewed interest: unlike at the time of its introduction, suitable instrumentation for measuring fluorescence polarization is now commonly present in many laboratories, for use in therapeutic drug monitoring (8). Numerous workers now consider this approach as useful as the L/S ratio.

Many obstetricians can assess FLM with a surprising accuracy simply by observing the specimen of amniotic fluid. Most mature specimens have a characteristic opalescent turbidity caused by the large number of lamellar bodies present at maturity. Lamellar bodies, which are the actual "packages" of surfactant in the amniotic fluid, are quite large (typically several micrometers in diameter) and thus scatter light intensely despite their relatively low mass concentration. Measuring absorbance at 650 nm ("O.D. 650") (9) attempts to quantify this clinical observation, and is perhaps the simplest and least expensive of all assessments of FLM. However, this method, like many others, suffers notably from pigment interferences (e.g., meconium, hemoglobin, methemoglobin, and methemalbumin) but is used occasionally. Although such interferences can be significantly reduced (10), the method remains on the periphery of testing modalities. Part of this hesitation to use improved methods for measuring absorbance at 650 nm, even in uncontaminated specimens, is perhaps based in part on the perception that a test that requires no reagents and is very simple to perform cannot be useful.

Very recently, methods have been advanced to enumerate the lamellar bodies themselves, either by differential spectrophotometry (10) or with electronic particle counters (11, 12). These approaches are very rapid, straightforward, objective, and inexpensive. Further, the use of electronic particle detectors is the only method in which a preliminary centrifugation or filtration is not required (11). Although they promise good correlation with other, more traditional approaches, these techniques require more evaluation and experience before their use can be recommended.

Because nearly all of the methods now used for FLM have very high predictive value for the nonoccurrence of RDS, it has been proposed that a "cascade" method (13) of testing might be an effective use of the laboratory. In this approach, one begins by performing one of the rapid tests (e.g., foam stability), proceeding to another test (with L/S by TLC being the third and last step) only if maturity is not predicted by either of the rapid methods. Garite et al. reported (13) that this approach could save time and expense both by using the rapid, inexpensive tests first, and by requiring that more than one test indicate immaturity, thereby increasing the predictive value of the immature result. Further, after two immature results by the rapid tests, the value for the L/S ratio will, even if also immature, give a good idea of what stage the pulmonary maturation has reached. Because L/S and PG are widely accepted as the definitive tests, however, such a cascade approach has not yet gained wide favor.

Although it is unlikely that the assessment of FLM will become less important in the near future, two related issues may eventually alter current attitudes toward testing. Recent years have brought very encouraging developments in the use of natural and synthetic surfactants in both the treatment of RDS and as prophylaxis in high-risk infants. Thus, at some time in the future, even when laboratory testing indicates pulmonary immaturity, fetuses may be more readily delivered pre-term should there be compelling reasons to do so. On the other hand, RDS is hardly the only risk associated with premature delivery and the risks of patent ductus arteriosus, intraventricular hemorrhage, and necrotizing enterocolitis, among many others, are still present even if an "ideal" laboratory test were to "guarantee" lung maturity.

What conclusions can we reach from this brief history of the problem? First, perhaps the time has come at least to begin to end the often heated discourse between laboratory and clinician regarding the "stat" and continuous availability of TLC. In most cases in which there is neither meconium nor blood pigment staining, the literature supports the valid use of many of the rapid tests, which do offer a high sensitivity and predictive value for the mature fetus. Progressing to other tests in such a setting is chiefly to reduce the number of false-positive results (immature results for mature infants) rather than to prevent RDS. Second, when staining is present, only the methods that assess PG can generally be considered reliable. Third, the L/S ratio determined by TLC has an important role in those cases in which the PG is shown to be absent by the rapid approaches. In such cases, L/S affords some additional information regarding the fetus' progress toward maturity, although the test could be performed in a more routine manner (instead of "stat") because the fetuses is less likely to be mature. Additionally, in a setting of increased amniotic fluid volume, the L/S ratio is unaffected by the dilution, and PG is similarly unaffected if determined as a ratio such as PG/S. Finally, we must accept that all of the currently available methods give results that offer high predictive value only if negative (mature); positive (immature) results possess low predictive value. This absence of the "perfect" test is likely to remain for the foreseeable future. Thus, when adopting strategies for assessment of FLM, it is appropriate that the issues of skill and labor intensiveness, cost, and speed associated with each test be given more consideration than in the past.

It is not productive to attempt rapid change in long followed approaches. Such change can come more readily if we work in a cooperative and gradual manner. Each medical center might proceed to evaluate rapid methods that are attractive to it. Initially, such testing might be offered without charge as an adjunct to the TLC so that physicians could become familiar and comfortable with the results so obtained. After a reasonable period, TLC could be offered only in specific indicated settings. With time and experience, these indications would become fewer and generally more manageable by the batched, routine mode in which most institutions could effectively perform TLC analysis of phospholipids in amniotic fluid. Such a policy together with the coming advances in perinatal medicine could end the quest at hand and preserve more time and resources for the many new and different challenges constantly before the laboratory.

References

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