

## Main clinical analyses on amniotic fluid

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**Abstract.** Background and aim of the work: To suggest a series of clinical analyses performable on amniotic fluid, in different medical complications occurring during pregnancy. Methods: Various methodology, for different obstetrics situations, were compared among them. Results: The main indication for late amniocentesis are the tests for fetal lung maturity. The most broadly accepted is the L/S ratio, which has become the "gold standard", although this chromatographic technique is labor intensive compared to several other procedures proposed and used. Conclusions: By amniocentesis, accurate fetal diagnosis has become possible. Clinical diagnostic tests are critical for many obstetric situations including premature rupture of membranes, management of pre-eclampsia, prevention of RDS. More recently, the widespread use of amniocentesis has led to study different aspects of fetal metabolism and/or fetal molecules whose functions remain to be established.

**Key words:** Amniotic fluid, amniocentesis, pulmonary maturity tests, PROM

Amniotic fluid analyses are used in a variety of medical complications occurring during pregnancy. The main indication for late amniocentesis (second to third trimester) is the determination of fetal lung maturity in obstetric situations including premature labor, premature rupture of the membranes, management of preeclampsia, elective caesarean section, fetal distress, suspicion of intrauterine growth retardation (IUGR), isoimmunization or diabetic pregnancy. Scarpelli (1) was the first to suggest that analysis of amniotic fluid (AF) phospholipids (PL) could provide an index of fetal lung maturity and of the risk of RDS. The disease, which occurs almost exclusively in premature infants, is caused by a surfactant deficiency, due to defect in the production of pulmonary surfactant by type II alveolar cells of the lungs; it is associated with a high mortality rate (30%-70%) and a significant risk of long term neurological or pulmonary sequel. The surfactant production (a complex composition of protein and phospholipids) increases with gestational age, markedly after 34-35 weeks of gestation,

preventing total alveolar collapse at the end of expiration. The amount of surfactant components in AF changes towards the end of gestation in a manner related to fetal lung maturity (2, 3). AF contains phospholipids (PL), including lecithin (L), sphingomyelin (S), phosphatidylinositol (PI), phosphatidylglycerol (PG) and phosphatidylethanolamine (PE), some enzymes of the pathways of PL synthesis, lamellar bodies, and lung specific apoprotein. The evaluation of all these components is the basis for a number of pulmonary maturity tests which may predict the presence or absence of respiratory distress syndrome (RDS) in the newborn infants.

They can be divided in:

- a) indirect surfactant tests (optical density at 650 nm, fluorescent polarization, thromboplastic activity);
- b) biophysical methods (the shake test, the foam stability index, surface tension measurement, bubble clicking, capillary flow rate, tap test, lamellar body count);

- c) biochemical methods to evaluate surfactant components and/or to determine surfactant component versus reference compound (lecithin/sphingomyelin, palmitate/stearate, phosphatidylglycerol/total phospholipid, saturated lecithin/total lecithin, phosphatidylglycerol/total phospholipid).

Each of them measures some aspects of surfactant contained in the AF and the turnaround time varies from 10 minutes to 4 hours. The ideal analyses of fetal pulmonary maturity should be rapid, economical, standardized and have a high predictive value for both immature and mature results. Nevertheless, no single test of amniotic fluid for prediction of fetal lung maturity has been yet found to be exclusively, completely reliable, easily performed and completely relevant in the prediction of fetal pulmonary maturity. The tests more frequently used for predicting fetal lung maturity include L/S ratio, phospholipid profile (PP), PG, foam stability test,  $A_{650}$ , tap test and lamellar body count.

*L/S ratio.* Gluck et al. (4) were the first to demonstrate that the AF lecithin to sphingomyelin ratio (L/S) is a index of fetal lung maturity; to date, is still the most widely used and accepted method. It takes 3-4 hrs or more to be performed. In normal pregnancies, L/S ratios correlate closely with gestational age. In many high-risk pregnancies with maternal diseases, however, there is not a good correlation, since biochemical maturation of the fetal lung may be either accelerated or delayed depending upon the maternal disease.

*The phospholipid profile (PP)* has been advocated as the most complete method for the evaluation of fetal lung maturity. This include the simultaneous determination of L/S, PG, PI and PE in AF is essentially not a more difficult method than the L/S ratio. However, two-dimensional chromatography is required since the one-dimensional method can be misleading.

*PG.* Chromatographic, enzymatic, and immunologic methods (5, 6) have been described for PG determination. The accuracy of PG is critically dependent on the assay method. Among the available tests, the most widely accepted remains PG using thin-layer chromatography. L/S and PG complement each other

in assessment of lung maturity. Furthermore, PG results are accurate and resistant to contaminants (blood or meconium) in AF, whereas L/S ratio may be altered.

PP, L/S and PG have time-consuming and cost limitations and require laboratory technician training and experience. The complexity and the need for special equipment limit the application of these tests to larger, more sophisticated laboratories. Therefore, a large effort has been made by investigators to develop other tests, based on the determinations of surfactant constituents in the AF, that might be quicker, easier to perform and useful as a screening method to ascertain fetal pulmonary maturity.

*Shake test* (foam stability test or Clements test) (7): A sample of AF is diluted serially with 95% ethanol and each sample is shaken. The test evaluates foam formed from the surfactant of AF.

*Optical density.* The presence of particles of surfactant suspended in AF increases the turbidity of the specimen. The absorbance at 650 nm increases late in gestation and correlates with the L/S and fetal lung maturity (8). The method rapid and simple, is affected by polyhydramnios, contamination, prolonged refrigeration and high centrifugation.

*Tap test:* 1 ml of AF in a test tube is added with one drop of 6N HCL and with 1.5 ml of diethyl ether, then tapped briskly three or four time (9). In the AF from mature fetus, the bubbles quickly rise to the surface and break down while in the AF from immature fetus the bubbles are stable or break down slowly. The Shake test and tap test are inexpensive, rapid and simple methods for the detection of surfactant-active material in AF. For obstetricians working in a peripheral hospital with limited access to a central biochemical laboratory, these test appear to be valuable procedures for predicting fetal pulmonary maturity.

*Lamellar body count.* Lamellar bodies are concentrically-layered particles secreted by type II pneumocytes into amniotic fluid; they consist almost entirely of phospholipids and represent the storage form of pulmonary surfactant (10). The particles are 1-5  $\mu\text{m}$  in diameter and their phospholipid content change with maturation of the fetal lung. Lamellar body count is obtained by analysing the AF sample with a cell counter.

## Clinical interpretation

None of the above mentioned methods is completely reliable: some infants develop RDS in spite a normal pulmonary maturity test, whereas others are free from the disease in spite of an "immature test". All methods have more false immature than false mature results. Determination of L/S is by far the most widely used and accepted method, although false mature values, (from 1% to 15%), in complicated pregnancies, can be found.

The additions of PG analysis is very useful in certain instances; in fact PG is more predictive of lung maturity than the L/S in pregnancies complicated by diabetes mellitus. Furthermore, when the PG is present, RDS does not occur even when the L/S is  $\leq 2$ . For obstetricians working in a peripheral hospital with limited access to a central biochemical laboratory, the tap test and shake test appears the only performable tests, but where laboratory facilities are available the PG analysis must be carried out.

### *Tests for PROM diagnosis*

Premature rupture of fetal membranes (PROM) occurs in 4.5%-7.6% of pregnancies. When occurring before 37 weeks of gestation it is associated with increased incidence of amnionitis and prematurity and increased fetal and maternal morbidity and mortality. Until few years ago, to ascertain the diagnosis of ruptured fetal membranes (PROM), in cases in which the diagnosis is clinically doubtful, the test widely employed was the amniotic fluid crystallization test. Recently, various proteins present in the amniotic fluid, including prolactin, alfa-fetoprotein, fetal fibronectin,  $\beta$ -HCG and IGFBP-1 (insulin-like growth factor binding protein-1) have been suggested as markers of amniotic fluid presence (11-14). The most promising test seems to be IGFBP-1. For its detection, in cervical/vaginal fluid, an immunochromatographic dipstick test, based on monoclonal antibodies has been developed. IGFBP-1 levels in amniotic fluid are 100-1000 times higher than in serum, thus its presence in vaginal sample indicates the presence of amniotic fluid, and is a clear indication of PROM.

Infants of diabetic mothers and those with eryth-

roblastosis fetalis after delivery frequently have the same metabolic and clinical findings at birth: mild or severe hypoglycemia, hyperinsulinemia and anatomic changes of pancreatic islets. Measurements of AF insulin concentrations (15) in diabetic pregnant women have been suggested as good indicators of fetal status because origin of AF insulin is supported by its inability to cross the placenta and its filtration in urine.

An other test, widely performed in amniotic fluid, is bilirubin assessment to predict the severity of fetal haemolytic disease. First, described by Liley (16) in 1961, it is based on bilirubin levels measured spectrophotometrically ( $\text{IOD } 450$ ), and plotted by gestational age. His chart, or a modified version (17) is still widely used. Although other strategies have been proposed (fetal blood sampling and/or ultrasonography and Doppler) (18), many guidelines even recommend serial amniocentesis in pregnancies at risk of fetal anemia.

The widespread use of amniocentesis has led to increased interest in the biochemical composition of AF to assess various aspects of fetal metabolism. A new field may regard oncofetal antigens, widely express in AF (19), whose functions remain to be established.

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