EDUCATIONAL COMMENTARY – DETECTING PREMATURE RUPTURE OF FETAL MEMBRANES

Educational commentary is provided through our affiliation with the American Society for Clinical Pathology (ASCP). To obtain FREE CME/CMLE credits click on Continuing Education on the left side of the screen.

Learning Objectives
Upon completion of this exercise, the participant will be able to:

- discuss the problems associated with premature rupture of fetal membranes.
- list and explain the tests used to detect rupture of membranes.
- describe the principle of the AmniSure ROM test (AmniSure International LLC, Cambridge, MA) for placental alpha-microglobulin-1 (PAMG-1).
- interpret AmniSure ROM results.

The sequence of events during normal labor is a progression from biochemical connective tissue changes in the cervix to uterine contractions and then to cervical dilatation. Spontaneous rupture of the fetal membranes (ROM) then occurs and is a normal component of labor and delivery. Prelabor or premature rupture of membranes (PROM) is defined as spontaneous leakage of amniotic fluid prior to the onset of labor and can occur at any gestational age. Preterm PROM (PPROM) is defined as PROM occurring prior to 37 weeks of gestation. The prevalence of PROM in term pregnancies is approximately 10%. Preterm PROM, the leading cause of preterm births, occurs in 20% to 40% of these births and accounts for approximately 18% to 20% of perinatal deaths in the United States. Fetal membranes serve as a barrier to ascending infection, and rupture of these membranes increases the risk of infection and can lead to other complications. Interventions following PROM include hospitalization, administration of corticosteroids and/or antibiotics, and induction of labor—all of which can also lead to complications. Because of these potential consequences, accurate methods for diagnosing PROM are needed.

Detection of ROM
Visualization of amniotic fluid pooling in the posterior fornix of the vagina is an inaccurate method for detection of PROM and is supplemented with use of one or more other tests. One of the most commonly used methods for detection of PROM is the nitrazine test, which confirms that the normally acid pH of cervicovaginal secretions is neutral or alkaline, presumably due to the presence of alkaline amniotic fluid. This test is associated with high false-positive rates and has been reported to have a specificity of 16% to 77%. Another commonly used test is the ferning test: microscopic observation of the distinctive crystallization pattern of dried amniotic fluid. Clinical diagnosis of ROM is usually obtained through the combined use of pooling, nitrazine, and ferning tests. Another chemical test that has been used is nile blue sulphate staining of fat-containing cells from fetal sebaceous glands, but these cells are present only after 32 weeks of gestation. All of the tests mentioned require speculum examination.

The "gold standard" for diagnosis of PROM is amnio-dye infusion into the amniotic cavity with confirmation of membrane rupture by detection of leakage of dye into the vagina (staining of a tampon) within 20 to 30
EDUCATIONAL COMMENTARY – DETECTING PREMATURE RUPTURE OF FETAL MEMBRANES (cont.)

minutes. This technique is highly invasive and may be associated with risk to pregnancy including bleeding, infection, iatrogenic rupture of membranes, and loss of pregnancy.

Biochemical Markers for PROM

The ideal biochemical marker for PROM would be a constituent present in high concentrations in amniotic fluid but absent in normal vaginal secretions, blood, and urine. One of the first such compounds tested was diamine oxidase, but the test is no longer available because it used toxic chemicals. Other markers that have been studied include alpha-fetoprotein (AFP), fetal fibronectin (fFN), prolactin, the beta subunit of human chorionic gonadotropin (β-hCG), urea, lactate, and creatinine. None of these markers demonstrated sufficient sensitivity and specificity to justify routine use for detection of PROM. Although fFN lacks the specificity to be used as a marker for PROM, it is useful in predicting the risk of premature labor in women between 24 and 36 weeks' gestation (see “Fetal Fibronectin,” API Chemistry Educational Commentary, 2004, 3rd test event).

Two proteins present in high concentrations in amniotic fluid are insulin-like growth factor binding protein-1 (IGFBP-1) and placental alpha-microglobulin-1 (PAMG-1). Rapid strip kits detecting these proteins have been developed and marketed for the detection of PROM. One of these tests, the Actim PROM test (Medix Biochemica, Kauniainen, Finland), a one-step membrane-immunoassay utilizing two monoclonal antibodies specific for IGFBP-1, is currently not available in the United States. The concentration of IGFBP-1 in amniotic fluid is up to 1000 times higher than that in maternal serum, and the rates of sensitivity and specificity for its detection range from 74% to 100% and 83% to 94.7%, respectively.

PAMG-1

Currently the only FDA-approved immunoassay for detection of membrane rupture is the AmniSure ROM test (AmniSure International LLC, Cambridge, MA), a one-step immunochromatographic assay using three monoclonal antibodies for the detection of PAMG-1. Placental alpha-microglobulin-1 is a 34-kd protein abundant in amniotic fluid (2,000-25,000 ng/mL) and present in very low concentrations (0.05-0.2 ng/mL) in cervicovaginal secretions. Concentrations of PAMG-1 <3 ng/mL may occur when vaginitis or an admixture of blood or serum is present but would not interfere with the test, which has a minimum detection threshold of 5 ng/mL. Significant discharge of blood may cause a false-positive result, and the manufacturer recommends that the test not be performed in the presence of such amounts of blood.

The sample used is cervicovaginal secretion taken by sterile swab inserted 2 to 3 inches with no speculum required. The test should be performed immediately after collection, but if this is not possible, samples can be stored refrigerated for 6 hours. The specimen is eluted into a vial containing solvent, and the test strip is then placed in the vial for approximately 5 minutes. Sample in the vial moves through the membrane and up the strip by capillary action and is exposed to the two zones on the pad: the test zone and then the positive control zone. The control zone contains anti-IgG, and if a visible line does not appear in this region, the test
EDUCATIONAL COMMENTARY – DETECTING PREMATURE RUPTURE OF FETAL MEMBRANES (cont.)

is invalid. The test zone contains anti-PAMG-1 antibodies, which form an antigen-conjugate complex visible as a line when PAMG-1 is present. Colloidal gold particles attached to the antibodies produce the colored lines. Thus, the presence of two lines (test and control) indicates rupture of membrane and one line (control zone only) indicates no rupture of membrane.

Initial studies indicated 99% accuracy, and a subsequent study confirmed ROM with a sensitivity of 99%, specificity of 88%, positive-predictive value of 98%, and negative-predictive value of 91%. When compared with the rapid strip test for IGFBP-1, the test for PAMG-1 was shown to be more sensitive.

Summary
The detection of amniotic fluid leakage and the diagnosis of PROM has relied on a combination of tests with relatively poor sensitivity and/or specificity. The development of assays for the detection of IGFBP-1 and PAMG-1 has provided rapid tests for detection of ROM. The test for PAMG-1 (Amni-Sure ROM) is the only such test available in the United States and has been shown to be both sensitive and specific for detection of amniotic fluid following rupture of the fetal membranes.

Suggested Reading
