Diagnosis of Premature Rupture of Membranes: Inspiration From the Past and Insights for the Future

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Abstract
Objective: To review the diagnostic methods described to confirm premature rupture of membranes during pregnancy, and to assess their effectiveness in establishing the diagnosis.

Data Sources and Extraction: The medical literature was searched to identify all relevant studies and reviews on methods for diagnosis of membrane rupture published in English up to January 31, 2009. Medline and the Cochrane databases were searched, and reference lists in identified articles were also examined. Articles not available through journals’ online editions were retrieved by manual search.

Study Selection: We identified 71 original studies and reviews on diagnostic methods of chorioamniotic membrane rupture published in English. These articles were reviewed and results were summarized based on the diagnostic test assessed.

Conclusion: Recognition of the importance of and difficulties in confirmation of rupture of the chorioamniotic membranes pervades past and present obstetric publications. The subjectivity and poor sensitivity of early diagnostic techniques for confirmation of ruptured membranes sparked technical advancements using biochemical markers. None of these biochemical tests have gained popularity, although novel techniques involving placental markers such as placental alpha microglobulin-1 may provide a future solution to the problem of diagnosing chorioamniotic membrane rupture.

Résumé
Objectif : Analyser les méthodes diagnostiques décrites pour confirmer la rupture prématurée des membranes pendant la grossesse, ainsi qu’en évalue l’efficacité pour ce qui est de l’établissement du diagnostic.

Conclusion : La reconnaissance de l’importance de la confirmation de la rupture des membranes chorioramniotiques et de ses difficultés est omniprésente dans les publications passées et présentes du domaine de l’obstétrique. La subjectivité, la faible sensibilité et les premières techniques diagnostiques visant la confirmation de la rupture des membranes ont donné lieu à des percées techniques faisant appel à des marqueurs biochimiques. Aucun de ces tests biochimiques ne s’est démarqué, et ce, malgré le fait que des techniques novatrices faisant appel à des marqueurs placentaires (comme l’alpha-microglobuline-1 placentaire) puissent fournir une future solution au problème que pose le diagnostic de la rupture des membranes chorioramniotiques.


INTRODUCTION

Premature rupture of membranes may occur at term or immediately preceding labour, or it may be an unexpected complication during the preterm period, when it is referred to as preterm premature rupture of membranes.

Chorioamniotic membrane rupture may have several underlying causes, although in many cases PROM and PPROM will not have recognized etiologies. The pathophysiology leading to PROM at term has been shown to be different from the pathophysiology leading to PPROM. At term, weakening of the membranes may result from physiologic changes combined with shearing forces induced by contractions.1–4 Generalized weakness of the membranes has been more difficult to identify with prematurely ruptured membranes.5 PPROM may result from a focal deficit rather than a generalized weakness of the membranes.6

Term PROM complicates approximately 8% of pregnancies.7 Among these, approximately 50% of affected women will begin labour spontaneously within 12 hours, 70% within 24 hours, 85% within 48 hours, and 95% within 72 hours.7–9 Fetal morbidities associated with term PROM include
ascending infection and in utero cord compression. Maternal risks of term PROM include chorioamnionitis and postpartum febrile morbidity. Preterm PROM, a complication of 2% to 20% of all deliveries, is a known important contributor to maternal and perinatal morbidity and perinatal mortality. Latency in PPROM, defined as the interval between PROM and birth, is known to be inversely related to gestational age at rupture, and is also related to a multitude of other factors, including number of fetuses, severity of oligohydramnios, myometrial thickness, and the existence of maternal or obstetrical complications. The major cause of perinatal morbidity and mortality associated with PPROM is prematurity. Morbidities related to prematurity include respiratory distress syndrome, necrotizing enterocolitis, interventricular hemorrhage, cerebral palsy, and sepsis. Other complications include in utero umbilical cord compression, cord prolapse and fetal distress, fetal malpresentation, placental abruption, chorioamnionitis with subsequent endometritis, and risk of operative delivery from this multitude of factors. Maternal sepsis is a rare but life-threatening complication reported in nearly 1% of cases. For over 70 years, there has been controversy among health care professionals about the optimal approach to clinical assessment and diagnosis of prematurely ruptured membranes. In most cases, membrane rupture can be confirmed by documenting amniotic fluid leakage from the cervical os with visualization of pooling in the posterior vaginal fornix. However, the diagnosis of PROM is difficult if there is a slow fluid leak or any bleeding, or when the classic “gush of fluid” does not occur. In addition, the relatively small amount of amniotic fluid present early in gestation further challenges the diagnosis of ruptured membranes. Laidors et al. showed that even later in pregnancy (after 34 weeks), speculum examination for visualization of amniotic fluid carries a 12% false negative rate when no fluid is seen. In 1951, Schuman described the “two sac” theory of membrane rupture: he stated that amniotic fluid can dissect between the two layers of membranes and produce a bulge that ruptures into the vagina, leaving one intact layer with remaining fluid enclosed and appearing to rupture at a later time. In such cases, women give a history of fluid leakage, and tests for leakage should be positive, although membranes appear clinically to be intact at delivery. Occasionally, women present with a history suspicious for membrane rupture, but clinical examination is negative or inconclusive; they subsequently return with clearly identifiable rupture of the membranes. Whether these cases represent preliminary minimal transudation of fluid across weakened membranes or minimal leakage around a firmly applied fetal presenting part cannot be determined. In approximately 20% to 25% of cases, rupture of membranes is not grossly apparent. A patient’s history may suggest membrane rupture, but test results are non-confirmatory, creating an obstetrical dilemma. Early and accurate diagnosis of membrane rupture would allow for gestational age-specific interventions to optimize perinatal outcome and minimize serious complications. Therefore, the search for an ideal test to diagnose membrane rupture definitively with no delay continues.

Although initial studies may be encouraging, diagnostic techniques are usually found subsequently to be limited by inaccuracies from false positives and false negatives with poorer sensitivities and specificities than originally anticipated. The ideal test should be simple, rapid, inexpensive, and non-invasive. Optimally, the accuracy of the test should not be hampered by the presence of blood, semen, infected urine, or other contaminants. An accurate biochemical marker for membrane rupture should have a high concentration in the amniotic fluid, a low concentration in maternal blood, and an extremely low background concentration in cervicovaginal discharge with intact membranes. We sought to review the diagnostic methods currently used to confirm premature rupture of membranes and to assess their effectiveness in establishing the diagnosis.

METHODS

We searched Medline and the Cochrane databases for all relevant studies and reviews published in English on accuracies of diagnostic methods for the diagnosis of rupture of the membranes up to January 31, 2009. Key words used for the Medline search were “premature rupture of membranes,” “amniotic fluid,” “diagnosis,” and “tests.” Articles not available online were retrieved by manual search. We found no Cochrane reviews of diagnostic methods of PROM, as discussed in this report. The studies identified showed evidence from levels II-2, II-3, and III, according to ranking of The Canadian Task Force on Preventive Health Care.

MICROSCOPIC FETAL CELL IDENTIFICATION

A literature review by Friedman et al. described the first microscopic technique for identification of fetal particles in amniotic fluid, developed by Philipp et al in 1929 and published in the German literature. In this technique, fetal lanugo hairs were identified in amniotic fluid. This was presumed to be incontrovertible evidence of membrane rupture when identified in vaginal secretions. However, because of the

ABBREVIATIONS

AFI: amniotic fluid index
PAMG-1: placental alpha-microglobulin-1
PROM: premature rupture of membranes
PPROM: preterm premature rupture of membranes
scant amounts of fetal lanugo hair in amniotic fluid and the fact that such hair was present in amniotic fluid only later in pregnancy, this method never gained popularity. As this study was not published in the English language, statistical data are not shown in the Table.

Subsequent diagnostic tests were based on cytologic inspection for fetal squamous cells in the vagina. Interest in cytologic diagnosis was based on the noted absence of stain within the cytoplasm and nucleus of vernix caseosa cells compared with vaginal squamous cells. Investigators searched for fetal cells in vaginal fluid using diverse stains including Masson stain, Sudan III to demonstrate fetal fat particles, Papanicolaou stain, pinacyanole stain, acridene orange stain, and Nile blue sulphate stain.

Recognizing the challenge to diagnose PROM in clinically equivocal cases, Averette et al. studied 100 patients in whom the integrity of the chorioamniotic membrane could not be determined by visualization of fluid on speculum examination. Fetal cell staining with pinacyanole to identify vernix caseosa cells in such cases showed high accuracy rates (97%), similar to those in earlier studies among patients with clinically confirmed membrane rupture. Although fetal cell staining techniques were considered rapid, simple, and durable, concerns about inaccuracies emerged. Contamination from the powder on examination gloves gave false positive results as anuclear granules mimicked fetal cells, and hypercornified vaginal cells also simulated anucleate fetal cells. False negatives resulted from the subjectivity of inspection, having insufficient cellular material, or after a prolonged time interval from membrane rupture. Accuracy rates appear to diminish with increased duration between presumed membrane rupture.

### Accuracy of diagnostic tests for PROM

<table>
<thead>
<tr>
<th>Test used for diagnosis</th>
<th>Discriminatory concentration</th>
<th>Sampling site</th>
<th>Patients, n</th>
<th>Mean (range)</th>
<th>SN, % (Mean (range))</th>
<th>SP, % (Mean (range))</th>
<th>FN, % (Mean (range))</th>
<th>FP, % (Mean (range))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal cells (bromthymol blue)</td>
<td>N/A</td>
<td>Vagina</td>
<td>239</td>
<td>(176–239)</td>
<td>90.2</td>
<td>98.4</td>
<td>7.5</td>
<td>1.7</td>
</tr>
<tr>
<td>pH</td>
<td>≥ 6.5</td>
<td>Vagina</td>
<td>125</td>
<td>(46–250)</td>
<td>90.2</td>
<td>79.3</td>
<td>12.4</td>
<td>28.2</td>
</tr>
<tr>
<td>Fetal cells (Masson stains)</td>
<td>N/A</td>
<td>Vagina</td>
<td>275</td>
<td>(50–100)</td>
<td>92.8</td>
<td>92.7</td>
<td>4.3</td>
<td>7.4</td>
</tr>
<tr>
<td>Fetal cells (Papanicolaou stain)</td>
<td>N/A</td>
<td>Vagina</td>
<td>75</td>
<td>(50–100)</td>
<td>92.8</td>
<td>92.7</td>
<td>4.3</td>
<td>7.4</td>
</tr>
<tr>
<td>Fetal cells (Pinacyanole stain)</td>
<td>N/A</td>
<td>Vagina</td>
<td>150</td>
<td>(100–250)</td>
<td>88.3</td>
<td>95.4</td>
<td>11.7</td>
<td>3.7</td>
</tr>
<tr>
<td>Fetal cells (Acridene orange stain)</td>
<td>N/A</td>
<td>Vagina</td>
<td>200</td>
<td>(100–300)</td>
<td>75.5</td>
<td>90</td>
<td>24.6</td>
<td>10.0</td>
</tr>
<tr>
<td>Fetal cells (Nile blue sulphate)</td>
<td>N/A</td>
<td>Vagina</td>
<td>106</td>
<td>(100–111)</td>
<td>89.5</td>
<td>98.6</td>
<td>10.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Crystallization</td>
<td>N/A</td>
<td>Vagina</td>
<td>198</td>
<td>(51–509)</td>
<td>90.8</td>
<td>95.3</td>
<td>4.4</td>
<td>4.7</td>
</tr>
<tr>
<td>History alone</td>
<td>N/A</td>
<td>N/A</td>
<td>100</td>
<td>90.3</td>
<td>88.4</td>
<td>9.7</td>
<td>11.6</td>
<td></td>
</tr>
<tr>
<td>History + pH + crystalization</td>
<td>N/A</td>
<td>Vagina</td>
<td>100</td>
<td>90.8</td>
<td>95.6</td>
<td>9.2</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>History + pH + Nile Blue stain</td>
<td>N/A</td>
<td>Vagina</td>
<td>100</td>
<td>87.1</td>
<td>92.7</td>
<td>12.9</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td>History + Crystallization + Nile Blue stain</td>
<td>N/A</td>
<td>Vagina</td>
<td>100</td>
<td>87.1</td>
<td>95.6</td>
<td>12.9</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>History + ultrasound + crystallization</td>
<td>N/A</td>
<td>N/A</td>
<td>83</td>
<td>85.1</td>
<td>78.6</td>
<td>11.1</td>
<td>21.4</td>
<td></td>
</tr>
<tr>
<td>pH + Crystallization + Nile Blue stain</td>
<td>N/A</td>
<td>Vagina</td>
<td>100</td>
<td>90.8</td>
<td>95.6</td>
<td>9.2</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>AmnioInjection Evans blue T-18</td>
<td>N/A</td>
<td>Amnio-centesis</td>
<td>18</td>
<td>100.0</td>
<td>100.0</td>
<td>0.0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>DAO</td>
<td>22.5 µU/L</td>
<td>Vagina</td>
<td>168</td>
<td>(100–272)</td>
<td>88.9</td>
<td>96.3</td>
<td>8.5</td>
<td>1.7</td>
</tr>
<tr>
<td>3.32 log glucose + log fructose</td>
<td>17.7279</td>
<td>N/A</td>
<td>215</td>
<td>98.9</td>
<td>96.0</td>
<td>7.7</td>
<td>2.6</td>
<td></td>
</tr>
</tbody>
</table>

continued
and diagnostic testing. Early authors including King and Bourgeois reported 57% and 81.8% accuracy rates, respectively, beyond the initial 24-hour period of membrane rupture. This was later confirmed by others, and shorter intervals to testing were shown to affect rates of diagnostic accuracy.

As novel methods were developed, older cytologic techniques began losing popularity, because they were time-consuming, required trained cytologists, were ineffective before 32 weeks’ gestation, and provided an uncertain diagnosis of membrane rupture.

LITMUS PAPER AND pH TESTING

Litmus paper testing began development at approximately the same time as fetal cell staining methods. Because of the difference in pH of vaginal secretions (4.5 to 5.5) and amniotic fluid (7.0 to 7.5), it was rightly assumed that the pH of vaginal secretions would rise when contaminated by escaping amniotic fluid. This assumption prompted preliminary experiments with bromthymol blue dye and, later, nitrazine applicators. Although similar in principle to bromthymol blue, a reported advantage of nitrazine is the complete change in colour of the applicator when exposed to amniotic fluid in vaginal secretions. Impregnated with

<table>
<thead>
<tr>
<th>Test used for diagnosis</th>
<th>Discriminatory concentration</th>
<th>Sampling site</th>
<th>Patients, n</th>
<th>SN, % (Mean)</th>
<th>SP, % (Mean)</th>
<th>FN, % (Mean)</th>
<th>FP, % (Mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolactin RIA</td>
<td>3mU/mL, 2ng/mL, 20.2µIU/mL</td>
<td>Vagina</td>
<td>57</td>
<td>88.0 (76.0–100.0)</td>
<td>85.0 (70.0–100.0)</td>
<td>68.0</td>
<td>15.0 (0–30.0)</td>
</tr>
<tr>
<td>AFP (RIA)</td>
<td>53.3 µg/L (2–125)</td>
<td>Vagina</td>
<td>122</td>
<td>80.6 (17.6–100.0), NR</td>
<td>91.9 (80.0–97.4), NR</td>
<td>5.4</td>
<td>8.1 (0.0–16.0), NR</td>
</tr>
<tr>
<td>AFP (RIA)</td>
<td>77.5 µg/L (30–125)</td>
<td>Cervix</td>
<td>89</td>
<td>92.0 (84.0–100.0)</td>
<td>86.9 (85.7–88.0)</td>
<td>8.0</td>
<td>13.2 (0–16.0)</td>
</tr>
<tr>
<td>human placental lactogen (hPL) RIA</td>
<td>31 ng/mL</td>
<td>Vagina</td>
<td>52</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>IFN monoclonal assay (ROM check)</td>
<td>50 ng/mL</td>
<td>Vagina</td>
<td>160</td>
<td>93.6 (90.0–98.2)</td>
<td>65.7 (26.8–97.0)</td>
<td>13.1</td>
<td>17.0 (3.0–25.0)</td>
</tr>
<tr>
<td>IFN monoclonal assay (ROM check)</td>
<td>25 ng/mL</td>
<td>Vagina</td>
<td>46</td>
<td>92.9 (85.7–92.9)</td>
<td>11.9 (74.4–100.0)</td>
<td>7.1</td>
<td>7.3 (0–39.2)</td>
</tr>
<tr>
<td>IGFBP-1 immunoenzyme (PromTest)</td>
<td>35 µg/L (3–100)</td>
<td>Vagina, cervix</td>
<td>130</td>
<td>84.9 (74.4–100.0)</td>
<td>92.8 (71.4–98.2)</td>
<td>14.7</td>
<td>7.3 (1.8–28.6)</td>
</tr>
<tr>
<td>IGFBP-1 dipstick (ActimPROM)</td>
<td>400 µg/L</td>
<td>Amniocentesis</td>
<td>20</td>
<td>98.8 (98.7–98.9)</td>
<td>93.8 (87.5–100.0)</td>
<td>1.2</td>
<td>6.3 (1.1–1.3)</td>
</tr>
<tr>
<td>Lactate</td>
<td>&gt;4.5 mmol/L</td>
<td>Vagina</td>
<td>200</td>
<td>86</td>
<td>92</td>
<td>92</td>
<td>87</td>
</tr>
<tr>
<td>hCG (ELCIA)</td>
<td>46.4 mIU/mL</td>
<td>Vagina</td>
<td>137</td>
<td>83.9 (68.0–100.0), NR</td>
<td>89.5 (84.2–95.0), NR)</td>
<td>9.1</td>
<td>10.5 (0.0–25.0)</td>
</tr>
<tr>
<td>Ultrasound amniotic fluid index</td>
<td>&lt; 80 mm/L</td>
<td>Amniocentesis</td>
<td>150</td>
<td>94.0</td>
<td>91.0</td>
<td>6.2</td>
<td>9.0</td>
</tr>
<tr>
<td>Creatinine concentration assay</td>
<td>0.36 mg/dL</td>
<td>Vagina</td>
<td>114</td>
<td>100.0</td>
<td>100.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Urea concentration</td>
<td>12 mg/dL</td>
<td>Vagina</td>
<td>139</td>
<td>100.0</td>
<td>100.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PAMG-1 (AmniSure™)</td>
<td>5 ng/mL</td>
<td>Vagina</td>
<td>194</td>
<td>98.8</td>
<td>93.8</td>
<td>1.2</td>
<td>6.3</td>
</tr>
<tr>
<td>PAMG-1 (AmniSure™)</td>
<td>5 ng/mL</td>
<td>Amniocentesis</td>
<td>20</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>AmnioSense™ absorbent pad</td>
<td>pH = 5.2</td>
<td>N/A</td>
<td>69</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

Legend: SN: sensitivity; SP: specificity; FN: false negative; FP: false positive; DAO: diamine oxidase; IGFBP-1: insulin growth factor binding protein-1; PAMG-1: placental alpha microglobulin-1; hCG: beta subunit human chorionic gonadotropin; IFN: fetal fibronectin; AFP: alpha fetoprotein; RIA: radioimmunoassay; NR: not reported; N/A: not applicable
sodium-dinitro-phenylozonaphol-disulphonate, nitrazine paper showed promising early results in detecting membrane rupture, with accuracies of 100%35 and 98.9%36 in clinically ruptured cases, and similarly high rates of accuracy among intact cases. This preliminary high optimism regarding nitrazine testing decreased by the 1960s,31,37 because of false intact cases. This preliminary high optimism regarding ruptured cases, and similarly high rates of accuracy among labour.43 Thus, the result of the fern test became viewed as rates of 21.2.% and 40.6%, respectively, in women not in tested for amniotic fluid crystallization, compared with rates of 11.8% and 2.0%, respectively, in labouring women not). These authors showed false positive and false negative populations (i.e., whether the women were in labour or published data is at least partly due to differences in study diagnostic reliability of the fern test compared with earlier semen, or cervical mucus.23,41,42

In most ferning specimens tested in early studies, 23,26,37–40 sensitivity and specificity analyses were performed on women in labour, in whom there was certainty about the diagnosis of membrane rupture.43 In most clinical situations, however, the test is intended for patients who have equivocal rupture. De Haan et al.44 showed that the modest diagnostic reliability of the fern test compared with earlier published data is at least partly due to differences in study populations (i.e., whether the women were in labour or not). These authors showed false positive and false negative rates of 11.8% and 2.0%, respectively, in labouring women tested for amniotic fluid crystallization, compared with rates of 21.2.% and 40.0%, respectively, in women not in labour.43 Thus, the result of the fern test became viewed as supportive rather than conclusive for non-labouring women with non-specific vaginal fluid loss.

**AMNIOTIC FLUID CRYSTALLIZATION**

Amniotic fluid crystallization, created primarily by the sodium chloride and protein content, began to dominate cytologic stains, with reported accuracies in clinically ruptured cases ranging from 73.0% to 98.5%,26,27,32,37–40 Screening for arborization provided an accuracy of 97.8% compared with 87.3% for litmus paper testing.37 Interestingly, Tricomi et al.37 suggested that fluid for examination should be aspirated from the vagina no further than 3 cm from the introitus to avoid contamination and false positive ferning results from cervical mucus lying in the posterior fornix. Initial optimism with low false negative rates changed as missed diagnoses in the presence of blood, meconium, or heavy leukorrhea became recognized.53 False positive results were subsequently attributed to fingerprints, semen, or cervical mucus.23,41,42

In the late 20th century, it was theorized that the sonographic identification of oligohydramnios would develop after membrane rupture,44 thereby facilitating diagnosis and subsequent management. Manning et al.45 initially described a technique for measuring by ultrasound the deepest vertical pool of amniotic fluid in patients with intrauterine growth restriction. This method was later used to assess membrane rupture; it was shown that ultrasound quantification of the deepest amniotic fluid pocket is of poor quality in confirming membrane rupture.44 No significant difference was found in the mean depth of amniotic fluid pocket between 100 patients with confirmed term PROM and 51 patients with intact membranes.44 Oligohydramnios may not be detected in patients with confirmed PROM, possibly because drainage may become intermittent or even stop once the presenting part descends and acts as a plug, preventing further drainage.44 Robson et al. suggested that a significant amount of amniotic fluid needs to drain rapidly and continuously for oligohydramnios to occur, especially because the fluid is replaced to varying degrees by the fetus.44 Erdemoglu et al.46 showed that a reduction in the four-quadrant AFI below 80 mm did not reliably identify cases of suspected membrane rupture by history with negative visualization of fluid by speculum examination. The measurement of AFI offers no advantage over measurement of a single vertical pocket of fluid in cases where ultrasound is used to evaluate possible membrane rupture.

Indeed, these authors showed a precipitous increase in error rate when the time interval between membrane rupture and sampling exceeded two hours.23 False negative tests were more commonly associated with prolonged rupture. None of the traditional procedures proved entirely satisfactory in isolation, but combinations of any three of the following produced a diagnostic accuracy of 93.1%23:

1. a positive history,
2. a positive nitrazine test,
3. fluid crystallization, or

**ULTRASOUND ASSESSMENT OF AMNIOTIC FLUID VOLUME**

In the late 20th century, it was theorized that the sonographic identification of oligohydramnios would develop after membrane rupture,44 thereby facilitating diagnosis and subsequent management. Manning et al.45 initially described a technique for measuring by ultrasound the deepest vertical pool of amniotic fluid in patients with intrauterine growth restriction. This method was later used to assess membrane rupture; it was shown that ultrasound quantification of the deepest amniotic fluid pocket is of poor quality in confirming membrane rupture.44 No significant difference was found in the mean depth of amniotic fluid pocket between 100 patients with confirmed term PROM and 51 patients with intact membranes.44 Oligohydramnios may not be detected in patients with confirmed PROM, possibly because drainage may become intermittent or even stop once the presenting part descends and acts as a plug, preventing further drainage.44 Robson et al. suggested that a significant amount of amniotic fluid needs to drain rapidly and continuously for oligohydramnios to occur, especially because the fluid is replaced to varying degrees by the fetus.44 Erdemoglu et al.46 showed that a reduction in the four-quadrant AFI below 80 mm did not reliably identify cases of suspected membrane rupture by history with negative visualization of fluid by speculum examination. The measurement of AFI offers no advantage over measurement of a single vertical pocket of fluid in cases where ultrasound is used to evaluate possible membrane rupture.

**INTRA-AMNIOTIC DYE INJECTION**

By 1970, amniocentesis with injection of dye to confirm amniotic membrane rupture had become a commonplace procedure; it was thought to be safe and had high patient acceptability rates.37 Prior to amniocentesis, intravenous injection of radioisotope was performed for placental localization, and the amnio-injection was performed under local
anaesthesia. Interestingly, the two reported disadvantages of the procedure at that time were related to difficulty in diagnosis in the presence of meconium-stained fluid and the possibility of neonatal skin staining for 48 hours after dye injection. Several types of stains have been reported for amnio-injections, with safety hazards reported only for methylene blue (see below).

Although ultrasonographically guided transabdominal instillation of indigo carmine dye (1 mL of dye in 9 mL of sterile normal saline) and observation for fluid passage transvaginally is designated an “unequivocal” diagnostic method for confirmation of membrane rupture, this invasive test carries increased maternal and fetal risk. Inherent risks of intra-amniotic dye injection include trauma, bleeding, infection, and preterm labour. While strengthening diagnostic certainty, a “negative dye test” may occur if the membranes seal after previous amniotic fluid leakage.

**GLUCOSE AND FRUCTOSE MEASUREMENTS**

Gorodeski et al. examined the difference between amniotic fluid and cervicovaginal mucus in their concentrations of solutes. During pregnancy, glucose and fructose are present in high concentrations in cervical mucus, with reported means of 240 mg/100 mL and 30.4 mg/100 mL, respectively. Amniotic fluid concentrations are noticeably lower, with mean concentrations of 39 mg/100 mL and 3.3 mg/100 mL, respectively. Gorodeski et al. showed that low values of glucose and fructose are found in amniotic fluid aspirated in a true case of membrane rupture. Best results are obtained with a calculated linear sum of the values (3.32 log glucose + log fructose). These authors reported no confounding by meconium or vaginal discharge.

This technique is fairly impractical in testing for membrane rupture, and further studies have not expanded upon differential carbohydrate gradients.

**MODERN METHODS**

Because of the limitations of available testing methods, investigators have sought alternative markers in vaginal amniotic fluid, such as prolactin, alpha-fetoprotein, beta-subunit of human chorionic gonadotropin, fetal fibronectin, diamin oxidase, lactate, creatinine, urea, and insulin growth factor binding protein-1, previously called placental protein 1. Interest in assessment of these markers stems from their high concentrations in amniotic fluid compared with normal vaginal secretions, but all require special laboratory equipment and training. Although these markers are useful for patients with intact membranes or unequivocal membrane rupture, they remain unpopular due to cost, testing complexity, and low test sensitivities in cases of equivocal rupture.

**PLACENTAL ALPHA-MICROGLOBULIN-1**

Initially isolated in Moscow in 1975, PAMG-1 has undergone recent evaluation for diagnostic testing in PPROM. This 34kDa placental glycoprotein is abundant in amniotic fluid (2000–25 000 ng/mL), with much lower concentrations in maternal blood (5–25 ng/mL). The protein is present in negligible amounts in cervicovaginal secretions with intact membranes (0.05–0.2 µg/mL). The 1000- to 10 000-fold difference in concentration between amniotic fluid and cervicovaginal secretions stimulated interest in a PAMG-1 immunoassay. Marketed as AmnioSense (AmniSure International, Cambridge, MA), the assay’s minimum detection threshold for PAMG-1 is 5 ng/mL, sufficient for 99% accuracy with minimal false negatives. PAMG-1 can be detected with as little as 0.25 µL of amniotic fluid in 1 mL of vaginal secretions. In the presence of blood or vaginitis, the background level of PAMG-1 can occasionally reach a maximum of 3 ng/mL. False-positive results with use of the AmnioSense assay seem very unlikely, although these may appear with increased use. Further, assay of PAMG-1 appears to be reliable over a wide range of gestational ages (11 to 42 weeks), and proved superior to conventional combined clinical tests involving visualization of fluid pooling in the posterior fornix, arborization, and nitrazine testing.

This test is currently available in Europe and was recently approved by the Food and Drug Administration for use in the United States. AmnioSense is a novel rapid, non-invasive bedside test that may be very helpful in diagnosis of difficult cases without visible leakage. Further studies are needed to assess the reliability of the test according to the time from membrane rupture.

**NON-INVASIVE ABSORBENT PAD**

Efforts to be able to confirm chorioamniotic membrane rupture with minute amounts of amniotic fluid have recently led to the development of the absorbent pad, AmnioSense. This 12 × 4 cm pad has a central strip that changes colour on contact with fluid with a pH > 5.2. After contact with urine, the strip reverts to its original colour when dry. This is due to the detachment of conjugate-based nitrazine molecules by the urine ammonium ions. AmnioSense has undergone cytotoxicity and skin irritation and sensitization testing, and it complies with the US Pharmacopoeia Guidelines.

In a study of 34 women presenting with suspected membrane rupture, the AmnioSense pad initially showed 100% sensitivity; overall specificity was 75%, but when women with bacterial
vaginosis or Trichomonas vaginalis were excluded from analysis, the specificity increased to 90%.15

In a recent study, Mulhair et al.78 compared the reliability of the absorbent pad test with a standard of amniotic fluid pooling in the posterior fornix on speculum examination in a cohort of 139 women. They found a specificity of 65.0% and a sensitivity of 98.3% for the AmnioSense pad. The two studies of the absorbent pad currently available15,78 suggest that a negative AmnioSense result indicates intact membranes in term and preterm gestations in 99% of cases. A positive result, however, suggests only a 70% chance of ruptured membranes, and thereby warrants confirmation or further investigations to identify infections.15,78

It remains unknown whether potential confounding substances such as semen, blood, or meconium may be distinguished from amniotic fluid by the AmnioSense pad test. As women with negative pad checks are unlikely to have ruptured membranes, this would imply decreased need for an uncomfortable and intrusive speculum examination.

SAFETY

Safety data on diagnostic testing for rupture of chorioamniotic membranes show potential adverse neonatal effects related to intra-amniotic administration of methylene blue.79,80 Several cases of neonatal hemolytic anemia and hyperbilirubinemia, including sepsis and neonatal death, have been reported after intra-amniotic injection of methylene blue.80 These cases involved injection of 3.5 mL of 1% methylene blue solution, resulting in doses of 30 to 5mg.80 Even at a lower dose of 10 mg, Cowett et al.79 reported a case of hemolytic anemia and hyperbilirubinemia requiring two exchange transfusions and phototherapy. These adverse effects could not be attributed to other causes of neonatal jaundice. The neonate’s skin was discoloured for the first few days of life and methylene blue was identified in the urine during the first day of life.

Intra-amniotic dye as a marker for confirmation of PROM also poses risk to the mother, including bleeding, infection, and preterm labour.48 It is also an invasive test used to assess a common complaint.

We were unable to identify any safety data related to the use of vaginal swabs, either with or without use of a speculum, in screening for chorioamniotic membrane rupture. Potential risks of swabs might include ascending infection. A non-intrusive method, the AmnioSense absorbent pad, may conceivably lead to negligible rates of infections and skin irritation. Neither of the two published studies testing absorbent pads report any complications from use of the product.

CONCLUSION

Early techniques for establishing the diagnosis of ruptured membranes included fetal cell staining, vaginal pH determination and amniotic fluid crystallization. Criticism of such methods emphasized subjectivity in interpretation, poor sensitivity, and numerous confounding factors cross-reacting with substances in the vaginal reservoir.

The absence of a non-invasive “gold standard” for the diagnosis of PROM has led to technically advanced biochemical markers. Despite improved diagnostic value in cases of unequivocal membrane rupture or intactness, biochemical markers lack the sensitivity and specificity required in equivocal cases. They have also not become popular, in part, because of cost and complexity in testing.

In the common clinical situation where a health care provider encounters a patient with possible ruptured membranes, diagnostic accuracy is the key to successful management and improved perinatal outcome. Nearly one quarter of patients do not present with overt clinical evidence of ruptured membranes. We believe that recent techniques involving placental markers such as PAMG-1 and the AmnioSense absorbent pad, with minute sampling required, non-invasiveness, rapidity of testing, and high accuracy rates, may provide a solution to the clinical challenge of diagnosing ruptured membranes.

REFERENCES


