Biological markers for the diagnosis and the prognosis of preterm premature rupture of membranes

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PPROM
Preterm Premature Rupture Of Membranes

- PPROM : implication for perinatal health

- Management
  - Positive impact on perinatal health
  - But Invasive treatment
  - Consequences of false positive and false negative results

- Effective diagnosis
  - Clinical
  - Biological

- Prognosis in cases of PPROM
  - Preterm delivery or infection?
  - Many biomarkers studied
  - Biomarkers used currently
PPROM: frequency and impact on prematurity

- PPROM: 3% of pregnancies
- 20 to 40% of the PROM
- 30 to 40% of preterm deliveries


PPROM, labor and infection
PPROM, labor and infection

Main cause of preterm birth

Chorionic cells

Rupture of membranes

Myometrial Contraction

Cervical maturation

Preterm Markers

Preterm birth

Proteins released

inflammation

Preterm delivery

infection – inflammation


PPROM, labor and infection
PPROM consequences

- Maternal complications: chorioamnionitis (2-8%), endometritis
- Perinatal complications = preterm birth
  - Perinatal mortality
  - Short term complications: Enterocolitis, bronchodyplasia, periventricular leukomalacia,
  - Long term outcome: cerebral palsy, sensorial deficit, mental retardation
- Infection = additional pejorative factor

Management in cases of PPROM (1)
no signs of infection

before 32-34 Weeks

Hospitalisation
Antibiotics
Maternal transfer
Tocolysis 24-48 hours
Corticosteroids
Search for infection

after 32-34 weeks

Hospitalisation
Antibiotics
Maternal transfer
Search for infection
Induction of labor at 34-36 weeks
Management in cases of PPROM (2)
use of a high CRP in decisions

Clinical Chorioamnionitis
1-2 % at admission

High CRP

Before 30-34 Wks

No tocolysis To gain time...

After 30-34 Wks
delivery

« delivery»

Diagnosis of PPROM: clinical

Verspyck E et Al. J Gynecol Obstet Biol Reprod. 1999
Diagnostic methods and prognostic criteria in the case of premature rupture of the membranes

History taking and clinical examination are often sufficient
80%

Sterile speculum examination: leakage of amniotic fluid from cervical os continued and expanded by the mobilization
Biomarkers: Quality required

- No « GOLD STANDARD » to confirm the diagnosis of rupture of membranes
- No perfect biomarkers
- Strictly specific molecule of the AF
  - Present at all gestational ages
  - Slow degradation
  - Easily detectable at low concentration
  - The test should be rapid and available 24 h a day

- Prevent false positive results
  - Contamination (blood, seminal liquid)

- Prevent false negative results
  - Rupture of membranes very early in the pregnancy
  - Disappearance of the marker
  - Concentration too low

Published results
methodological drawbacks

Verspyck E, Landman T, Marpeau L J Gynecol Obstet Biol Reprod 1999

- No Gold standard for diagnosis of PPROM
- Very few studies compared diagnostic value of different tests
- Cut-offs to define an abnormal test could be different in the studies
- Comparison groups: No rupture of membranes versus cases with clear liquid flow
  Target population: suspicion of PPROM (intermittent or no leakage of fluid) - cases in which clinical diagnosis is not evident
Localization of the biomarkers used

amniotic fluid crystallization testing – fern test

<table>
<thead>
<tr>
<th></th>
<th>Sens</th>
<th>Spec</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fern test – Gibbs, 1982</td>
<td>42%</td>
<td>76%</td>
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Contamination with cervical mucus (false positive result)
Learning required (microscope)
subjective
A lot of biomarkers studied

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<tr>
<td>DAO (Diamine Oxidase)</td>
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<td>- Gaucherand, 1997</td>
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<td>- De Meeus, 1997</td>
<td>71</td>
<td>90,9</td>
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<td>100</td>
<td>98,3</td>
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<td>AFP (Alpha Feto-Protein)</td>
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<td>- Kishida, 1995</td>
<td>103</td>
<td>100</td>
<td>97,4</td>
<td>92,5</td>
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<tr>
<td>- Gaucherand, 1995</td>
<td>131</td>
<td>88</td>
<td>84</td>
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<tr>
<td>Others: hCG, Prolactin, urea, creatinin, lactates ...</td>
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<tr>
<td>amniotic fluid/serum ratio low</td>
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FIBRONECTINE

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<td>94</td>
<td>97</td>
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<tr>
<td>- Rutanen, 1993</td>
<td>54</td>
<td>92</td>
<td>80</td>
<td>79</td>
<td>-</td>
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Extracellular matrix protein of membranes > AF
Released in the case of preterm labor or labor with intact membranes
Protein present in seminal fluid
amniotic fluid/serum ratio low

NITRAZINE paper testing
simple, cheap

cervical pH = 5-6
Amniotic fluid: alkaline

Dipstick colorimetric

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<tr>
<td>- De Meeus, 1997</td>
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<td>81,1</td>
<td>83,3</td>
<td>52,6</td>
<td>96,1</td>
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</table>

bacterial vaginosis
cervicitis
semen, alkaline urine,
blood, soap and
antiseptic solutions

false positive

IGFBP-1 (ActimProm®)

insulin-like growth factor binding protein-1
Produced by decidual cells (placental protein)
Immunoenzymometric assay for quantitation
Concentrations = 100 to 1000 fold higher than those in serum
Very low concentration in urine, cervical mucus and seminal fluid
Rapid strip test (5 min) = > 95th percentile of serum levels

<table>
<thead>
<tr>
<th>ActimProm – IGFBP-1</th>
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<td>96,5</td>
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<td>Marcellin et al. 2011</td>
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<td>95,3</td>
<td>95,6</td>
<td>97,7</td>
<td>93,6</td>
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</table>
PAMG-1 (AmniSure®)

placental alpha-microglobulin-1
Produced by decidual cells (placental protein)
Concentrations = 1 000 to 10 000 fold higher than those in cervical mucus

<table>
<thead>
<tr>
<th>Amnisure – PAMG-1</th>
<th>Nb</th>
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<td>Cousin et al. 2005</td>
<td>203</td>
<td>98,9</td>
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<td>99,1</td>
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<td>92,8</td>
<td>91,6</td>
<td>90,7</td>
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Prognosis in case of PPROM

Preterm birth

Prognosis of what?

Infection
Neonatal?
Maternal?

Neonatal outcome?
Long term outcome?
Intra uterine inflammation or infection

Clinical chorioamnionitis:
Admission: 1-2 %
Subsequently: 3-8 %

Positive culture by amniocentesis: 25-40 %

Neonatal infection: 5-10 %

Natural history of PPROM
Relationship between PROM and infection
cause and consequence

- **Chorio-decidual Infection or inflammation**
  - **Non infectious causes**
  - **1/2 ?**
  - **PPROM**
  - **materno-fetal infection and delivery**
  - **Delivery without infection**
  - **Most Women Proportion ?**

**Best way : diagnosis of inflammation or infection in amniotic fluid (amniocentesis)**

- Culture could be the gold standard for the diagnosis of infection
  - Direct diagnosis of the inflammation or the infection
  - Identification of the germa

- Many short term tests studied
  - Gram stain, white blood cell count, leucocyte esterase, glucose concentration
  - Interleukines, metalloproteinase
  - Etc etc
Disadvantages of amniocentesis

- Disadvantages of the procedure
  - Results of the culture (48 hours) after the delivery frequently
  - Short term test: low specificity and meaning?
  - Success of amniocentesis is associated with amount of amniotic fluid remaining (oligohydramnios in PPROM)
  - Complications associated with amniocentesis?

- No evaluation of the use of test
  - Decision?: antibiotics, Cesarean section, corticosteroid before extraction

- Few team published (many, many) papers on the diagnostic value of theses tests, many methodological weaknesses

Cytokines in serum or vaginal secretions?

- Parturition: IL-6, TNFα, IL-1β, IL-8

- Glycoproteins involved in inflammation
  - released mainly after activation of macrophages
  - result in the production of prostaglandins and proteases (MMP)
  - measurement difficult and costly (ELISA in most studies)

- Prospective study (73 women with PPROM) (Kayem et al 2005)
  - diagnostic value of IL-6 in vaginal secretions for neonatal infection in cases of PPROM
  - Immunochromatographic bed side test (20 minutes)
Odds of neonatal infection as function of maternal markers, based on logistic regression

<table>
<thead>
<tr>
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<th>Congenital proven or probable sepsis</th>
<th>Without congenital sepsis</th>
<th>OR</th>
<th>Adjusted OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal IL6 positive</td>
<td>12 (75.0)</td>
<td>29 (42.6)</td>
<td>4.0 (1.3-13)</td>
<td>5.5 (1.2-17.7)</td>
</tr>
<tr>
<td>Maternal serum C- Reactive Protein</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>&gt; 5 mg/dl</td>
<td>9 (52.9)</td>
<td>31 (41.3)</td>
<td>1.2 (0.5-4.6)</td>
<td></td>
</tr>
<tr>
<td>&gt; 20 mg/dl</td>
<td>4 (23.5)</td>
<td>5 (7.4)</td>
<td>3.4 (1.0-16.5)</td>
<td>5.6 (0.95-32.6)</td>
</tr>
<tr>
<td>Maternal WBC (x 1000 :)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>&gt; 15</td>
<td>3 (18.9)</td>
<td>21 (30.9)</td>
<td>0.6 (0.13-1.97)</td>
<td></td>
</tr>
<tr>
<td>&gt; 20</td>
<td>1 (6.2)</td>
<td>4 (5.9)</td>
<td>1.0 (0.1-10.2)</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion

- **Diagnosis**
  - **Clinical**
  - **Biomakers necessary in 10-20 %**
    - Nitrazine + PROM test or Amnisure test
    - Perspective: Biomarker strictly specific of the AF and easily detectable at low concentration
  
- **Prognosis**
  - **To predict subclinical infection before onset of labor, but**
    - Which kind of management?
    - No simple biomarkers available