Streptocoque groupe B  
Nouveaux tests de dépistage: PCR  
Perspectives vaccinales  

Centre National de Référence pour Streptocoque agalactie  
Microbiologie clinique, CHU de Liége, Université de Liége

INTRODUCTION & BURDEN

Group B streptococcal diseases in neonates

- Since the 1970s, leading cause of life-threatening infections in newborns
- Neonatal illness/death
- Long-term disabilities

EOD: 0.3-3 per 1,000 live births

LOD: 0.4-0.5 per 1,000 live births

GLOBAL health major challenge! Also in developing low income countries

Stages in the pathogenesis of GBS neonatal EOD: Bacterial & individual factors

INTRODUCTION
Early- and Late-onset GBS Diseases, U.S. WHEN?
Highly Incidence births (1989-2008) Expected high predictive values:

- False negative ➔ Missed IAP
- “False” positive ➔ Unnecessary IAP

WHY?
Process of GBS screening: To predict GBS vaginal (rectal) colonization at the time of delivery

Screening for GBS colonization

Screening for GBS colonization OLD & NEW TOOLS

Impact of prevention practices Early- and Late-onset GBS Diseases, U.S. Introducing:

CDC draft

Universal screening

Improved screening method

Late-onset GBS

Early-onset GBS

No effect on GBS EOD

Intraperitoneal antibioprophylaxis > 4 hours before delivery

Highly effective in preventing GBS EOD (1st clinical trials in late 80s)

European strategies for prevention of GBS EOD

• Intrapartum antibioprophylaxis recommended
  • Screening-based
  • Herd immunity
  • Incidence to 0.3-1 per 1,000 live births

- Spain, 2003, revised 2010
- Germany, 1996, revised 2015
- Switzerland, 2007
- Belgium, 2003, revised 2015
- France, 2001

Guarantees: Selective or active screening

Impact of prevention practices Risk-based strategy

Screening-based strategy

European strategies

¿WHY?¿

¿WHEN?¿

¿HOW?¿

Impact?

Screening for GBS colonization

Goal of GBS screening

To predict GBS vaginal (rectal) colonization at the time of delivery

Expected high predictive values:

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Screening for GBS colonization OLD & NEW TOOLS

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**Screening for GBS colonization**

**Goal of GBS screening**
*To predict GBS vaginal (rectal) colonization at the time of delivery*

- **Critical factors influencing accuracy**
  - Swabbed anatomic sites (distal vagina + rectum)
  - Timing of sampling
  - Screening methods (antenatal vs intrapartum)
    - Culture
    - Media
    - Nucleic Acid Amplification Test (NAAT)

**Antenatal culture-based screening: Limiting factors**

- **Positive and negative predictive values**
  - False-negative results
    - Failure of GBS culture
    - Reduced viability during transport, oral ATB, feminine hygiene
  - Up to 1/3 of GBS positive women at time of delivery

*Eagerly expected, a more accurate predictor for intrapartum GBS vaginal colonization*

**Detection of EOD risk = GBS positive colonization at delivery**

**Antenatal screening**
- VPP 60-87%
- VPN 88-96%
- False negative: missed IAP
- False positive: unnecessary IAP

**Intrapartum screening**
- Expected PPV and NPV >90%
- Better targeted IAP
- No susceptibility testing

**Prevention strategy for GBS EOD**
*TOWARDS A EUROPEAN CONSENSUS?*

- Conference held in June 2013, Florence, Italy

A European working party: Neonatologists, obstetricians, microbiologists

Representing countries:
- with screening-based IAP
- with risk-based IAP strategies
- or nothing
Towards « European Consensus »
Decision taken by the European working party

Main recommendations

- Universal screening at time of delivery
  - POCT with high PPV and NPV
  - Real time PCR or other methods
  - TAT < 1 hour
- IAP for all GBS positive pregnant women
  - Documented by intrapartum testing (or late pregnancy test if performed)
- Late pregnancy antenatal screening in known penicillin allergic women
  - Determination of clindamycin susceptibility if GBS positive screening


Test Xpert GBS

- Real Time PCR on GeneXpert system (Cepheid).
  - Amplification of a conserved region adjacent to the cfb gene of GBS
  - On vaginal or vagino/rectal swab
  - Fully automated
  - Easy handling
  - Result in 45 minutes

Material and methods

Specimen collection
Test Xpert GBS

Ongoing study in CHU Liège/UZ Antwerp: Objectives (→ 900 patients)

1. To assess the practical and analytical aspects of the implementation of the PCR test Xpert GBS® in Belgium
   • Performed by midwives
   • For all women at onset of labor
2. To evaluate the cost-effectiveness of the intrapartum screening strategy
   → To consolidate the proposal of the European Expert Group

Specimen collection

Prenatal screening
- vagino/rectal specimen collected at 35-37 weeks’ gestation

Intrapartum screening
- vaginal specimen using a double swab
  - From ALL women at onset of labor

Culture
Test Xpert GBS
Test Xpert GBS: Procedure

- Procedure performed by midwives
- GeneXpert system installed at the Obstetrics facility

Test Xpert GBS: Expression of results

Indeterminate status for GBS
Negative for GBS
Presence of GBS

Test Xpert GBS: Use of results

- Algorithm proposed to clinicians:
  - Integration of the intrapartum Xpert result in addition to:
    - Patient’s clinical data
    - Result of the antenatal screening at 35-37 weeks’ gestation

Preliminary results

Culture results
PCR results

Global overview

- Study period: 8/4 au 03/10/2014 (still ongoing)
- 658 deliveries
- Included patients: 486 Xpert® GBS tests performed (74%)
  - Inclusion rate lower among antenatally positive screened patients.

Culture results

- Colonization rate (35-37 weeks): 19.4%
- Performances of the antenatal culture screening

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>67.3%</td>
<td>94.2%</td>
<td>68.8%</td>
<td>93.8%</td>
</tr>
</tbody>
</table>
PCR results

- Not yet available for presentation
- Difficulties encountered:
  - Wrong manipulations
  - Invalid results
- Study still ongoing, with a revised protocol

Xpert® GBS for intrapartum testing (main papers)

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year/journal</th>
<th>Nb patients</th>
<th>Site</th>
<th>S %</th>
<th>Sp %</th>
<th>PPV %</th>
<th>NPV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mueller et al.</td>
<td>2014 Eur J Obstet Gynecol Reprod Biol</td>
<td>180</td>
<td>Lab</td>
<td>85.7</td>
<td>85.7</td>
<td>95.6</td>
<td>95.7</td>
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<td>Abdelkafi IIA</td>
<td>2013 Eur J Obstet Gynecol Reprod Biol</td>
<td>275</td>
<td>Lab</td>
<td>90.5</td>
<td>90.5</td>
<td>92.3</td>
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<tr>
<td>Peltier et al.</td>
<td>2013 Ann Lab Med</td>
<td>125</td>
<td>Lab</td>
<td>96.4</td>
<td>96.4</td>
<td>98.7</td>
<td>98.7</td>
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<tr>
<td>Chuchel EL et al.</td>
<td>2012 Diag Microbiol Infect Dis</td>
<td>250</td>
<td>Lab</td>
<td>100</td>
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<tr>
<td>De Tejada BM et al.</td>
<td>2011 Clin Microbiol Infect Dis</td>
<td>945</td>
<td>Obst.</td>
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<td>Young RC et al.</td>
<td>2011 Eur J Obstet Gynecol Reprod Biol</td>
<td>500</td>
<td>Lab</td>
<td>97.4</td>
<td>97.4</td>
<td>99.7</td>
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<tr>
<td>El Helali N et al.</td>
<td>2009 Clin Infect Dis</td>
<td>948</td>
<td>Lab</td>
<td>96.6</td>
<td>96.6</td>
<td>98.7</td>
<td>98.7</td>
</tr>
</tbody>
</table>

Discussion

- Bias linked to low inclusion of antenatally positive detected women
- 100% inclusion rate is utopian:
  - Delay before delivery too short, high workload
  - Technical problems, lack of involvement in the study.

Intrapartum PCR: Handling

- Test easy to perform « a priori » BUT...
- Many difficulties encountered by midwives:
  - Sample preparation
  - Proper breaking into the cartridge
  - Loading in the instrument
- Large team, high turn-over

  → Continuous training required

Conclusion (1)

- Intrapartum screening:
  - Proven clinical value
  - Recommended by new European directives
  - Cost-effectiveness remains to be demonstrated
- Test Xpert GBS:
  - Sensible et specific
  - Fully automated
  - Fast result
  - Feasible in point-of-care, 24h/24
  - Easy to perform...
  BUT...
Conclusion (2)

**Necessary supervision by the lab:**
- Careful training of operators
- Verification of test performance before routine implementation
- Daily technical supervision
- Involvement of gynecologists:
  - ensure adequate inclusion rates
  - integrate the result of the rapid test in the care of the patient

Conclusion (3)

**Is the Xpert® GBS test enough robust to be universally recommended as a POCT?**

**Desired developments at Cepheid:**
- Internal control checking for human cells
- Simplifying the interface of the GeneXpert system

Today GBS is still the leader!

- GBS remains leading cause of EO sepsis & meningitis
  - Up to 60% of occurring among women with negative antenatal screening
  - Highlighting limitation with screening and IAP
  - IAP has no effect on incidence of GBS LOD

Maternal GBS immunization

- Protection against both EOD & LOD?
- Bypassing concerns related to antimicrobial resistance?
- Cost-effectiveness?
- Adjunctive to screening & IAP?

**History of vaccine development**

MATERNAL IMMUNIZATION

**CONTENT**
- Maternal immunization
- History of development
- Different antigens / options
- Where are we today?
- Different vaccines
- Cost-effectiveness
- Take home messages
**Streptococcus agalactiae or GBS**

- Gram positive cocci
- β-hemolytic
- Encapsulated
- 10 capsular serotypes (Ia, Ib, II-IX)
- Numerous surface proteins (α and β-C, rib, Sip, pilus islands 1, 2a & 2b, etc)

**Distribution (%) of capsular types of GBS isolated in neonatal disease**

- (DEVANI project, 2008-2011, EU Fund FP7 programme)

<table>
<thead>
<tr>
<th>Capsular Type</th>
<th>Distribution (%)</th>
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<tbody>
<tr>
<td>Ia</td>
<td>0</td>
</tr>
<tr>
<td>Ib</td>
<td>10</td>
</tr>
<tr>
<td>II</td>
<td>20</td>
</tr>
<tr>
<td>III</td>
<td>30</td>
</tr>
<tr>
<td>IV</td>
<td>40</td>
</tr>
<tr>
<td>V</td>
<td>50</td>
</tr>
<tr>
<td>VI-VIII</td>
<td>60</td>
</tr>
<tr>
<td>NT</td>
<td>70</td>
</tr>
</tbody>
</table>

- 76 neonatal EOD; 72 neonatal LOD

**Background**

- Long-standing data supports protection of maternal anti-CPS Ab

- **Lancefield’s observations**
  - Demonstration of protection against lethal GBS infection in a mouse model by antibodies to the CPS of GBS
  - Passive transfer of anti-CPS Ab protects newborn mice

**Maternal vaccination allows infant protection**

- **Correlate between maternal low level of CPS type Ab (III, Ia & Ib) at time of delivery and risk for development of GBS EOD**

- **Human serum containing sufficient concentrations of Ia, Ib, II, III and V CPS-specific IgG promotes efficient opsonization & phagocytosis of homologous strain in vitro and protection from experimental infection in vivo.**

  *Baker C et Kasper D, 1976, NEJM*

**Vaccine for pregnant women:**

- Likely the most effective, sustainable and cost effective approach
Background

First generation of CPS vaccine

- Disappointment from studies of uncoupled first generation purified native GBS CPS vaccines in healthy adults
- Demonstration of feasibility of vaccine prevention of GBS disease
- Need for improvement of immunogens
- Success story of polysaccharide-protein conjugate vaccine technology in preventing Hi b and S.pneumoniae infections in infants

Background

- Expectation of polysaccharide-protein glycoconjugates
- T cell-dependent response
- Immunological memory & long term protection
- Predominantly IgG1 subclass → improved transplacental transport
- Increase likelihood of protection of mother and infant

GBS Vaccines, since the 1980s Challenges

Native capsular polysaccharide vaccines (1st gen)

- 10 serotypes
  - Different distributions
  - EOD, LOD, invasives infections in adults
  - Geographically, along time, ATB pressure

Conjugated vaccines (2nd gen)

(Channing laboratory, Harvard medical school, Boston)

- CPS III-Tetanus Toxoid
- Monovalent Ia, Iib, II and V CPS –TT
- Tested for immunogenicity in healthy adults
- Multivalent conjugated vaccines Ia, Iib, (II), III (and V)

GBS Vaccines, since the 1980s Challenges

Capsular polysaccharide - TT vaccines
Capsular polysaccharide – CRM197 vaccines

(Second generation)

- Dosage and route of administration
- Immune response
- Duration of immunity and protection
- Safety studies
GBS Vaccines, since the 1980s

**Challenges**

- **Ag** = Surface proteins
  - Cross protection against different serotypes
- **Better immunogenicity**
  - Humoral response T-cell dependent
  - Long lasting immunity

**GBS Protein-based Vaccine**

- Alpha-like proteins
  - Yes (Ia, Ib et II)
- Alp1
  - Yes (Ia)
- Rib
  - Yes (III)
- Alp2
  - Yes (V, VIII)
- Alp3
  - Yes (V, VIII)
- Beta C protein
  - Yes (Ib)
- C5a peptidase
  - Yes (All)
- Sip
  - Yes (All)
- BPS
  - Yes (All)

Sip = Surface Immunogenic Protein (Brodeur, Martin, Quebec)

**BPS** = Groupe B Protective surface Protein

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Protein-based Vaccines

- **Reverse vaccinology approach**
- **Knowledge of complete GBS genome**
- **Comparison of genomes from 8 different GBS serotypes** (Novartis)
  - 312 surface proteins were cloned
  - 4 provide a high protective humoral response in mouse
  - Sip and 3 others
  - The 3 other proteins = « pilus like structures »
    - PI 1, PI 2a & 2b

**GBS « pilus like structure »**

- Highly immunogenic proteins
- Elicit protective and functional (opsonophagocytosis) antibodies
- Virulence factor
  - Adhesion
  - Transcytose through cells

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Protein-based Vaccines

- **GBS-NN fusion protein**

  - Based on novel vaccine epitopes identified in the N-terminal regions of the Rib and AlphaC surface-proteins of GBS
  - Vaccine candidate is a non-glycosylated fusion protein

**GBS-NN fusion protein**

*From Rib and AlphaC surface proteins of GBS*

- Highly Immunogenic

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GBS « pilus like structure »

- Immunodominant Repeats
- Non-immunodominant

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Protein-based Vaccines

- **GBS-NN Fusion protein**

  - Non-reducing interactions with antibodies
  - Highly Immunogenic

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Cell Host & Microbes 2: 427-434, 2007
**Protein-based Vaccines**

**GBS-NN fusion protein**

- Based on novel vaccine epitopes identified in the N-terminal regions of the Rib and AlphaC surface-proteins of GBS
- Vaccine candidate is a non-glycosylated fusion protein
- Highly immunogenic and anti-GBS-NN antibodies more protective than antibodies to full-length proteins

A novel protein-only, single component, GBS vaccine covering 95% of clinical isolates

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**Protein-based Vaccines**

Anti-GBS-NN antisera prevents GBS invasion of epithelial cells

- Rib: III
- p = 0.001
- Ib
- p = 0.001

**Potential implications for pathogenesis and prevention of invasive disease by mucosal anti-NN IgG**

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**Novartis GBS Vaccine**

- **Trivalent glycoconjugate vaccine**

- CRM conjugated CPS Ia, Ib and III
- Trivalent conjugate coverage: 79% globally
- Phase I completed, and Phase II ongoing
  - Phase III study: EU/US/Global
    - Size: >10,000 mothers → >10,000 infants

**Planned start 2015**

- Eligibility: women between 28-35 wks gestation
- End-points: Mother infant safety; vaccine immunogenicity (efficacy); infant response to CRM-containing vaccines

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**Minervax GBS Vaccine**

- **Single component NN fusion protein**

- Anticipated coverage: 95% of isolates
- Clinical trial in healthy adults: Q2-2015
- EU funding FP7 Programme HEALTH for the development of a novel innovative GBS vaccine candidate
- Other sources of funding
- Phase 1 study will start in UK (announced 2 June 2015)
Comparison and middle

As an important

strategy

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GBS vaccine Phase III trial

Importantly,

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CONCLUSION
Take home messages

GBS vaccine - Conclusion

- CPS-glycoconjugate vaccine
  - 3 to 5-valent glycoconjugate vaccine (Ia, Ib, II, III and V)
- CPS-CRM197 / Pili vaccine
- NN-fusion protein vaccine

- Immunogenicity
- Safety
- Efficacy determination ongoing
- Impact on colonization: unknown

Maternal GBS immunization

Conclusion

- Immunization at 28-32 weeks
- Prevention at least 85% of invasive GBS disease in neonates and young infants
- Potential reduction
  - of incidence of maternal invasive GBS infection
  - of premature births, stillbirths related to GBS infection
- Cost-effective in high and low income countries

Thank you for your attention!