Introduction

Screening for GBS colonization

Old and new tools

Antibiotic resistance

Threat to therapy?

Maternal immunization

Take home messages

Prevention of GBS disease

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Clinical Microbiology, University Hospital of Liege, University of Liege

INTRODUCTION & BURDEN

Streptococcus agalactiae or GBS

Gram positive cocci

β-hemolytic

Encapsulated

10 capsular serotypes (Ia, Ib, II-IX)

Streptococcus agalactiae or GBS

Group B streptococcal diseases in neonates

Since the 1970s, leading cause of life-threatening infections in newborns

Neonatal illness/death

Long-term disabilities

1887, Noccard-Mollereau, bovine mastitis

1933, Group B Antigen

1964, severe neonatal sepsis, Eickhoff et al. N Engl J med

1970, N°1 in neonatal infections

Streptococcus agalactiae clones infecting humans were selected and fixed through the extensive use of tetracycline

Genome-based phylogeny reveals the expansion of a few clones

Human GBS belong mainly to a small number of TcR clones

V.Dacunha, MR.Davies, ... C.Puyart and P.Glaser

Group B streptococcal diseases in neonates

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

> 80% EOD

A. Schuchat, Clin Microb Rev 1998;11:497-513

Stages in the pathogenesis of GBS

**neonatal EOD:** Bacterial & individual factors

- **Colonization:** adhesion to epithelial cells different virulence factors (pili, scpB, ...)
- **Preventing transmission**
- **Intrapartum antibioprophylaxis** > 4 (2) hours before delivery
- Highly effective in preventing GBS EOD (1st clinical trials in late 80s)

GBS vaccine « nearly within reach »

Help for clearing bacteria and preventing development of EOD

Screening for GBS colonization

**OLD & NEW TOOLS**

**WHY?**

**WHEN?**

**IMPACT?**

**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 birth

**LOD**

0.4-0.5 per 1,000 live birth

GLOBAL health major challenge
Also in developing countries

**Stages in the pathogenesis of GBS**

**neonatal EOD:** Bacterial & individual factors

- Colonization: adhesion to epithelial cells different virulence factors (pili, scpB, ...)
- Preventing transmission
- Intrapartum antibioprophylaxis > 4 (2) hours before delivery
- Highly effective in preventing GBS EOD (1st clinical trials in late 80s)

GBS vaccine « nearly within reach »

Help for clearing bacteria and preventing development of EOD

Screening for GBS colonization

**OLD & NEW TOOLS**

**WHY?**

**WHEN?**

**IMPACT?**
OBJECTIVES
To provide a comprehensive picture of current and coming practices for GBS screening
Culture methods versus NAAT
Antenatal versus intrapartum

To emphasize critical criteria for success
To identify some possibilities for improvement
To point out advantages and drawbacks

No guidelines

Critical factors influencing accuracy
To predict

Swabbed
Anatomic sites (distal vagina vs. rectum)
Timing of sampling
Screening methods
Culture
Procedure
Media
Nucleic Acid Amplification Test (NAAT)

Screening methods
Timing of sampling

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

Screening
Risk-based strategy

Universal screening

Intrapartum

Critical conditions to optimize
universal antenatal SCREENING

WHEN: 35-37 weeks
WHO: ALL the pregnant women
Specimen: Vaginal + rectal swab(s)
Collection: WITHOUT speculum
Transport: Transport/collection device/condition (non nutritive medium: Amies/Stuart or Granada like tube) (type of swab) (length and T°)
Request form: To specify prenatal « GBS » screening
Laboratory procedure

European strategies for prevention of GBS EOD

Intrapartum antibiotic prophylaxis recommended
Screening-based strategy

• Spain, 1998, 2003, revised 2012
• France, 2001
• Belgium, 2003, revised 2015
• Germany, 1996, revised 2008
• Switzerland, 2007

Risk-based strategy

• UK, the Netherlands, Denmark

No guidelines

Screening for GBS colonization

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

Expected high predictive values

False negative
Missed IAP

“False” positive
Unnecessary IAP

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 vs. rectum)
• Timing of sampling
• Screening methods
  • Culture
  • Procedure
  • Media
  • Nucleic Acid Amplification Test (NAAT)

Swabbed
Anatomic sites (distal vagina vs. rectum)
Timing of sampling
Screening methods
Culture
Procedure
Media
Nucleic Acid Amplification Test (NAAT)

Crucial conditions to optimize
universal antenatal SCREENING

WHEN: 35-37 weeks
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Laboratory procedure

CDC draft
1992

Universal screening

Before national prevention policy
No effect on GBS LOD

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

Before national prevention policy
No effect on GBS LOD

Incidence of early- and late-onset invasive group B streptococcal disease in sentinel Active Bacterial Core surveillance areas, 1989-2008 (CDC 2010)

Universal screening

Incidence of early- and late-onset invasive group B streptococcal disease in sentinel Active Bacterial Core surveillance areas, 1989-2008 (CDC 2010)

CDC’s 1st consensus guidelines

Screening – Risk-based

Screening

CDC draft
1992

Early-onset GBS

Late-onset GBS

CDC’s 1st consensus guidelines

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CDC’s 1st consensus guidelines

Screening – Risk-based

Screening
Optimal time for screening
35-37 weeks gestation

Culture-based screening done 1 to 5 or > 6 weeks before delivery (Yancey, 860 cases; Melin, 532 cases)

Not 100% as colonization is dynamic


Remaining burden of GBS EOD
Missed opportunities
In spite of universal screening prevention strategy
In spite the great progress
Cases still occur

• Among remaining cases of EOD
  • Some may be preventable cases
  • Missed opportunities for (appropriate) IAP

DEVANI project, unpublished data 2011

Which agar or which combination?
+/- Blood agar

Workload - costs - extra-testing - non β-hemolytic GBS detection to be considered

Crucial conditions to optimize SCREENING

• WHEN
  35-37 weeks

• WHO
  ALL the pregnant women

• Specimen
  Vaginal + rectal swab(s)

• Collection
  WITHOUT speculum

• Transport
  Transport/collection device/condition (non nutritive medium: Amies/Stuart or Granada like tube) (type of swab) (Length and T°)

• Request form
  To specify prenatal GBS screening

• Laboratory procedure

(CDC 2010 - Belgian SCV 2012)
Crucial conditions to optimize SCREENING

Transport-collection system & storage-transport condition
- Type of swab: Nylon flocked >> regular fiber swab

Results:
Recovery of GBS in Lim BD at 4°C, RT and 35°C (Clinical studies ongoing)

- Recommendations CDC, USA (2010)
  - Non nutritive media: Amies or Stuart without charcoal
  - Storage at 4°C or RT 1-4 days
    - Or Granada like tubes ??
- Recommendations CSS, Belgium (2003)
  - Non nutritive media: Amies or Stuart without charcoal
  - Storage maximum 48h at 4°C

Use of a selective enrichment Lim broth: Recommended by some European guidelines
- To avoid overgrowth of other organisms
- To maximize the isolation of GBS

Transport conditions to be recommended for optimizing GBS antenatal screening
Belgian Health Superior Council, 2015

- Transport system
  - Use of a selective enrichment Lim broth with a flocked swab (BD, Copan, bioMérieux, i.e.)
- Transport and storage condition
  - At RT° (up to 35°C)
  - As soon as possible
    - Viability sustained at least 4 days
- Remark
  - If use of Amies or Stuart medium (non nutritive medium)
    - To be processed as soon as possible within 24 hours (max 48 h)

Remark
Non nutritive media: Amies or Stuart without charcoal
- Maximum 48h at 4°C

To sustain viability
Whatever is storage T° for a few days
Use of a selective enrichment Lim broth as transport media

Continuous decrease

Important amplification
Antenatal culture-based screening combined with amplification molecular test

NAAT performed from Lim enrichment broth Broth
- The Xpert GBS LB assay
- The LAMP illumigene GBS Assay

Clinical evaluations
- Speed: time to result minus one day
- Accuracy: good comparison to reference culture
- Cost, logistic, training: very important to consider

Evaluation of the illumigene® GBS assay for antenatal screening from Lim broth

CHU Liege & UZ St Lucas, 2012
- Speed and “accuracy”
- Good comparison to reference culture method
- “Easy” to perform BUT not as easy as claimed and training very important
  - 90% → 95% sensitivity (PCR)
  - 100% specificity
  - Identification of an 0.8% additional GBS positive specimen
  - Overall cost and logistic to be considered

Alternative to GBS antenatal screening: intrapartum screening

Theranostic approach

Turnaround time
- Collect specimen at admission
- Optimal management of patient
- 30-45 minutes, 24 hrs? 24 h broth

Results
- Sensitivity > 90%
- Specificity > 95%

Intrapartum screening theranostic approach

Expected advantages: pro & con
- Inclusion of women without prenatal screening/care
- Identification of women with change of GBS status after 35-37 wks gestation (new acquisition, false negative)
- Increased accuracy of vaginal GBS colonization status at time of labor & delivery
- Drawback: no antimicrobial susceptibility result

IAP addressed to right target
- Reduction of inappropriate/unnecessary IAP
- Broader coverage of « at GBS risk women »
- Improvement of prevention
Real Time PCR for intrapartum screening

- Advance in PCR techniques & development of platforms & to be used as a POCT
  - BD GeneOhm™ Strip B Assay (+/- 1 hr) (in laboratory)
  - Xpert® GBS, Cepheid (35-45 min) (can be performed as a POCT)

Xpert® GBS for intrapartum screening

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

Xpert® GBS for intrapartum screening (selected paper among many others)

Cost and effectiveness of intrapartum group B streptococcus polymerase chain reaction screening for term deliveries.
El Helali N, Giovangrandi Y, Guyot K, Chevet K, Gutmann L, Durand-Zaleski I

Obstet Gynecol 2012 Apr;119 (4):822-9

2009
Antenatal screening
11.7% GBS POS

Xpert GBS intrapartum screening
16.7% GBS POS
Less GBS EOD & less severe

Cost neutral per delivery


Required analytical specification for rapid intrapartum test

- High sensitivity and specificity
  - Minimum 90% and 95% respectively
- Full automation with integrated internal controls
- Easy to perform and interpret by nurses
- Time to result: < 1 hour
- 24 h / 7 days availability

Xpert® GBS for intrapartum screening (selected paper among many others)

Diagnostic Accuracy of a Rapid Real-Time Polymerase Chain Reaction Assay for Universal Intrapartum Group B Streptococcus Screening
Najwa El Helali, Jean-Claude Nguyen, Aïcha Ly, Yves Giovangrandi and Ludovic Trinquart

Clinical Infectious Diseases 2009;49:417–23

968 Pregnant women
- Intrapartum Xpert GBS, Cepheid (performed in lab)
- vs intrapartum culture

Antenatal culture
(Renal swab/CNA-BA)

- Sensitivity 98.5%
- Specificity 99.6%
- PPV 97.8%
- NPV 99.7%
- PPV 58.3%
- NPV 92.1%

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

Lim & Sub-Culture
- aGranada, bStrepB Select, cJLGa-CAAX

Test Xpert GBS

Test Xpert GBS: Procedure
- Procedure performed by midwives
- GeneXpert system installed at the Obstetrics facility

Test Xpert GBS: Results

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
  - Sensitivity: 67.3%
  - Specificity: 94.2%
  - PPV: 95.8%
  - NPV: 93.8%

intrapartum culture as gold standard
PCR results

- « Not yet available »
- Difficulties encountered:
  - Wrong manipulations
  - Invalid results
- Pause of the study and revision of protocol

Real Time PCR for intrapartum screening

- Advance in PCR techniques & development of platforms & to be used as a POCT
- Xpert® GBS, Cepheid (35-45 min)
  Already recommended by CDC for women with no prenatal care, …

- Easy BUT …
- Midwives teams: numbers, turn-over
- TRAINING is essential
  - Sample preparation
  - Proper breaking the swab into the cartridge
  - Loading the instrument
  - To be used under lab control

Xpert® GBS for intrapartum screening

(main papers)

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Journal</th>
<th>Nb patients</th>
<th>Site</th>
<th>S %</th>
<th>Sp %</th>
<th>PPV %</th>
<th>NPV %</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
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<tr>
<td>Mueller al</td>
<td>2014, ERL / Obstet Reprod Biol</td>
<td>Lab &amp; Obst</td>
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<td>Poncelet et al</td>
<td>2013, BJOG</td>
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<td>2013, Aust NZ Obstet Gynaecol</td>
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<td>Park et al</td>
<td>2013, Ann Lab Med</td>
<td>Lab</td>
<td>175</td>
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<td>Church et al</td>
<td>2011, Diag Microbiol Infect Dis</td>
<td>Lab</td>
<td>231</td>
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<td>De Tejada et al</td>
<td>2011, Clin Microbiol Infect</td>
<td>Obst</td>
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<td>Young et al</td>
<td>2011, Am J Obstet Gynaecol</td>
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GBS and Antibiotic Resistance

Threat to Therapy ?
**Background**

- **Intrapartum antimicrobial prophylaxis (IAP)**
  - Penicillins = first line drugs
  - In case of IgE mediated allergy (risk of anaphylaxis)
  - Clindamycin, if susceptible
  - Vancomycin, if clindamycin resistant or unknown status

- **Treatment of infections**
  - Penicillins = first line drugs
  - +/- combination with aminoglycosides in severe infections
  - According to site of infections
    - Macrolides, clindamycin, fluoroquinolones

**Antibiotics and GBS in 2014**

- **Penicillins**
  - GBS still fully susceptible to P and most β-lactams
  - Very rare non S GBS (Japan, USA, Canada, ... ?)
  - Macrolides and lincosamides
    - R on the rise
    - 5 – 35 % R, even more to erythromycin and clindamycin
  - Geographical differences
    - High level resistance reported (up to 13% in Argentina)

- **Gentamicin**
  - Increasing for a decade, mainly in Japan, Korea, China (up to 37%)
  - FLQ on the rise

Will penicillins remain the gold standard?

**GBS and non-S to β-lactams**

- Existence and molecular mechanisms of clinical isolates with reduced Penicillin susceptibility (PRGBS)
  - First report in Japan by Kimura K et al, AAC 2008
  - Following reports from Japan, USA, Canada
    - Penicillin MIC 0.25-1 mg/L
    - Ceftizoxime MIC 4-128 mg/L
    - Acquisition of amino-acid substitutions in PBP2X and in PBP1A
    - Elevation of cephalosporins/MICs

**PR GBS versus PR S.pneumoniae**

- **PR S.pneumoniae**
  - Penicillin MICs increased by acquiring various amino-acid substitutions in PBPs, including PBP1A and PBP2X
  - Why should we not see the same in GBS?
    - Risk of highly resistant cephaporin GBS
    - Risk of increase of MICs to penicillin

**PR GBS detection**

- Possibly unrecognized by standard antimicrobial susceptibility methods !!
  - Recommended methods for initial screening
    - 3-Disk diffusion
    - Oxacillin, ceftizoxime, Ceftibuten (no zone)
    - MICs to oxacillin and ceftizoxime
    - Usually high for PR GBS

Kimura et al, J Clin Microbiol 2009
What do we know today about macrolide - lincosamide Resistance?

Resistance to macrolides/lincosamides
Wide geographical variation of rates

Resistance to macrolides/lincosamides on the rise (Invasive isolates of GBS Belgium 1999-2012)

MLS acquired Resistance
Phenotypes and genotypes

- Target modification (erm family genes)
  - Constitutive MLS resistance
  - Inducible MLS resistance (D-zone test)
  - Serotype associated (higher rates: IV, V > III > others)
  - Cross resistance to macrolides, lincosamides and streptogramin B
- Active efflux (mefA gene) → M phenotype
  - Resistance to 14- & 15-membered ring macrolides (as erythromycin and azithromycin)

Distribution of erythromycin R genes
(French GBS NRC 2007 – 2011)
Distribution of erythromycin R genes among invasive isolates (Belgian GBS NRC, 2012)

**Distribution of Erythromycin R Genes among Invasive Isolates**
- MLSc: 37.02%
- MLSi: 4.85%
- MLSc: 8.13%
- MLsi: 64.93%

**MLS Acquired Resistance Phenotypes and Genotypes**
- Target modification (erm family genes)
- Constitutive MLS resistance
- Inducible MLS resistance
- Cross resistance to macrolides, lincosamides and streptogramin B
- Active efflux (mefA gene)
- Resistance to 14- & 15-membered ring macrolides (as erythromycin and azithromycin)

**Enzymatic inactivation or ? (Inu genes, Isa genes)**
- Clindamycin resistance

**Phenotypes L**
- L phenotype
  - Inactivation by lincosamide nucleotidytransferases (Inu(B) and Inu(C) genes)
    - New Zealand, Canada, USA, Asia, Argentina
  - LS, or LS,P phenotype
    - Cross resistance to lincosamides, streptogramin A and pleuromutilin
    - Isa(C) gene
      - New Zealand (Malbruny et al., AAC, 2011)
      - Belgium (J.Descy et al, LISSSD abstract 100)
    - 0.6% from 223 isolates (2008-2012)

**Aminosides**
- Emergence of high-level resistance to gentamicin and streptomycin in Streptococcus agalactiae in Buenos Aires, Argentina
- 141 GBS from vagino-rectal swabs
  - HLR Gentamicin: 13.5%
  - HLR Streptomycin: 16.3%
- Detection methods
  - Disks GEN (120 µg) and STR (300 µg)
  - MICs to GEN and STR (Etests)
  - Agar screening plates (GEN 100mg/L; GEN 500mg/L; STR 2000 mg/L)
- Very rarely reported in Europe

**Fluoroquinolones**
- Emerging fluoroquinolone resistance in Streptococcus agalactiae in South Korea
  - 221 GBS from pregnant women + 838 patients
  - R unexpectedly high:
    - Ciprofloxacin 9.3%; Levofloxacin 9.5%; Moxifloxacin 0.8%
    - Mutation detected in gyrA and topoisomerase genes
    - +/- 4% in Belgian isolates
- 37.7% Levofloxacin 37.7%
  - 80% belonged to GBS ST19/serotype III clone with GyrA-ParC-ParE triple substitution
  - this clone carrying erm and mef genes
- Clonal expansion of multi-drug-resistant GBS → concerns about its future spread

**Fluoroquinolones**
- High prevalence of Fluoroquinolone-resistant Group B Streptococci among clinical isolates in China and predominance of sequence type 19 with serotype III
  - Huai Wang et al, AAC, 2013:1538-41
  - 146 GBS from different locations in China, 2011
  - Levofloxacin 37.7%
  - 80% belonged to GBS ST19/serotype III clone with GyrA-ParC-ParE triple substitution
    - this clone carrying erm and mef genes
    - Clonal expansion of multi-drug-resistant GBS → concerns about its future spread
**Tetracycline Resistance**

- The most frequent antimicrobial resistance marker
- > 85% of GBS isolated from human infection

Streptococcus agalactiae clones infecting humans were selected and fixed through the extensive use of tetracycline

By V. Dacunha, M.R. Davies, ..., C. Poyart and P. Glaser


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**GBS Antibiotic Resistance: where are we going?**

**EPILOGUE**

- Emergence of resistance is unavoidable
  - But how fast?
- Transmission of Resistant genes « in package »!
  - Risk of increase of multi-resistance
  - Threat for both prophylaxis and therapy
- Emphasize the need for
  - careful epidemiologic monitoring
  - good clinical laboratory AST practice

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**Antibiotics**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>About Resistance</th>
<th>Epidemic surveillance by Nat.Ref.C.</th>
<th>AST - Routine lab methods</th>
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<tbody>
<tr>
<td>Penicillin and other β-lactams</td>
<td>Still very rare – Possibly unrecognized</td>
<td>Mandatory</td>
<td>Initial screening by with 3-disks diffusion To implement in clinical labs worldwide?</td>
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<tr>
<td>Gentamicin</td>
<td>Emerging in some countries – Not routinely screened</td>
<td>Mandatory</td>
<td>HLR determination for severe infections Method ???</td>
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<tr>
<td>Fluoroquinolones</td>
<td>Emerging in Asia – Rare elsewhere</td>
<td>Mandatory</td>
<td>No special trick</td>
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**Maternal GBS immunization**

Could maternal immunization be an alternative?

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

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**History of vaccine development**

MATERNAL IMMUNIZATION
Background

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

Background

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 la, 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

Background

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

Background

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 \(Hb\) and \(S.pneumoniae\) infections in infants

Maternal vaccination allows infant protection
- Placental transfer increases markedly \(> 32\) weeks

Vaccine for pregnant women:
Likely the most effective, sustainable and cost effective approach

CANDIDATE VACCINES

CPS
Conjugate CPS
Surface proteins
Pili proteins
NN fusion protein
GBS Vaccines, since the 1980s
Challenges

Native capsular polysaccharide vaccines (1st gen)
- 10 serotypes
- Different distributions
  - EOD, LOD, invasive infections in adults
- Geographically, along time, ATB pressure

Conjugated vaccines (2nd gen)
(Channing laboratory, Harvard medical school, Boston)
- CPS III-Tetanus Toxoid
- Monovalent Ia, Tb, II and V CPS – TT
- Tested for immunogenicity in healthy adults
- Multivalent conjugated vaccines Ia, Tb, (II), III and V

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

Protein-based Vaccines

<table>
<thead>
<tr>
<th>Protein</th>
<th>Protective Ab</th>
<th>associated serotypes (in mouse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-like proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha</td>
<td>Yes</td>
<td>Ia, Ib et II</td>
</tr>
<tr>
<td>Alp1</td>
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<td>Ia</td>
</tr>
<tr>
<td>Rib</td>
<td>Yes</td>
<td>III</td>
</tr>
<tr>
<td>Alp3</td>
<td>Yes</td>
<td>V, VIII</td>
</tr>
<tr>
<td>Alp3</td>
<td>Yes</td>
<td>V, VIII</td>
</tr>
<tr>
<td>Beta C protein</td>
<td>Yes</td>
<td>Ib</td>
</tr>
<tr>
<td>CsA peptidase</td>
<td>Yes</td>
<td>All</td>
</tr>
<tr>
<td>Sip (1999)</td>
<td>Yes</td>
<td>All</td>
</tr>
<tr>
<td>BPS</td>
<td>Yes</td>
<td>All</td>
</tr>
</tbody>
</table>

Sip = Surface Immunogenic Protein (Brodeur, Martin, Quebec)
BPS = Groupe B Protective surface Protein

D.Malone et al, Science 2006
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

Comparison of genomes from 8 different GBS serotypes (Novartis)
Reverse vaccinology approach
Knowledge of complete GBS genome
- PI 1, PI 2a & 2b
GBS « pilus like structure »

Protein-based Vaccines

GBS-NN fusion protein
From Rib and AlphaC surface proteins of GBS

- 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

Rib and AlphaC surface proteins of GBS

GBS-NN Fusion protein

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

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

GBS-NN fusion protein

From Rib and AlphaC surface proteins of GBS

- 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

Vaccination with GBS-NN protects against lethal challenge with GBS Ia, Ib, II & III in adult mice

Mice immunized with GBS-NN in alum, boosted after 4 weeks and challenged 2 weeks later.

Strong clinical correlation exists between naturally occurring maternal and neonatal levels of anti-Rib and anti-Alpha antibodies

Strong correlation exists between levels of neonatal anti-Alpha (OR 0.0007) and anti-Rib (OR 0.002) and invasive GBS infection

Anti-GBS-NN more protective than antibodies against full length Rib and Alpha in animal models

Arch Dis Child Fetal Neonatal Ed 81:F403-408, 2006

Cell Host & Microbe 2, 427-434, 2007

Cell Host & Microbe 2, 427-434, 2007
Potential implications for pathogenesis and prevention of mucosal disease by mucosal anti-NN IgG

Figure 4. The N-Terminal Part of the

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

GBS Maternal immunization

Would it be cost-effective?

- Cases prevented,
- Deaths averted,
- Life-years saved
- Quality-adjusted life-years (QALYs) gained
- Costs of
  - Acute care for infants with GBS disease
  - Chronic care for those with long term disability
  - Immunization per person
- Assuming 85% coverage
  - Prevention of an additional 899 cases of GBS and an additional 35 deaths among infants in the US

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: (EUUS/Global)
    - Size: >10,000 mothers → >10,000 infants
  - Planned start 2015
  - Eligibility: women between 28-35 weeks gestation
  - End-points: Mother/Infant safety; vaccine immunogenicity (efficacy); infant response to CRM-containing vaccines

GBS Maternal immunization

Would it be cost-effective?

Prevention of group B streptococcal disease in the first 3 months of life: Would routine maternal immunization during pregnancy be cost-effective?

- Vaccine
  - Volume 02, Issue 37, 28 August 2014, Pages 4076-4079
GBS Maternal immunization
Would it be cost-effective?

In conclusion
Routine maternal immunization with a trivalent (Ia, Ib and III) vaccine at week 28 of pregnancy
- As an adjunct to screening and IAP
  - May address an important unmet public health need in the US
  - And further reduce the burden of GBS disease during infancy (EO and LOD)
- May be comparable in cost-effectiveness to several other vaccines recently approved to use in children and adolescents

GBS Maternal immunization
Would it be cost-effective?

- In low and middle income countries:
  - no screening-based IAP strategy
  - +/- RF-based IAP strategy
  - Comparison of 4 strategies
    - Doing nothing
    - Maternal GBS vaccination
    - RF-based IAP
    - Maternal GBS vaccination + RF-based IAP
  - Assuming 55-90% coverage and 75% of women vaccinated
    - Vaccination / Doing nothing → prevents 30-54% of cases
    - RF-based IAP / Doing nothing prevents 10% of cases
    - Vaccination + RF-based IAP → prevents 48% of cases
  - Substantial reduction of the burden of infant GBS disease in South Africa and would be cost-effective by WHO-guidelines

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

GBS VACCINE
CONCLUSION
Take home messages

Trivalent (Ia, Ib and III) glycoconjugate vaccine
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!