Baltimore, Congenital Syphilis, and Reverse Sequence Testing: Lessons Learned

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Learning Objectives

At the end of this presentation, the participant will be able to:

1. Discuss the challenges of diagnosing congenital syphilis infections, particularly in high incidence STI community.
2. Describe the techniques of reverse sequence (RS) testing for syphilis, and
3. Understand the development and application of an expert-derived algorithm for evaluation and management of congenital syphilis (CS) in an RS testing environment.

Treponema pallidum

- Macroaerophilic Gram negative bacteria
- Humans are the natural host
- Genome sequenced in 1998
- Outer membrane mostly consisting of lipids and few integral membrane proteins
- Metabolism is limited to host- T. pallidum has very few intrinsic enzymes
- The bacteria cannot be cultured on artificial medium

Early Congenital Syphilis: Clinical Manifestations

- Hydrops fetalis
- Intrauterine growth restriction
- GI
  - Hepatomegaly/hepatitis
- Hematologic:
  - Lymphadenopathy, anemia, thrombocytopenia
- Macrocysternum:
  - Edema, rash (pemphigus syphiliticus), rhagades
- Orthopedic:
  - Osteochondritis, periostitis
  - Wimberger's sign
  - Rhagades of Parrot
- Renal:
  - Nephrotic syndrome
- Ocular:
  - Chorioretinitis
- CNS
  - Leptomeningitis, chronic meningovascular syphilis (hydrocephalus, CN palsies, vascular infarctions)

Late Congenital Syphilis: Clinical Manifestations

- Dental:
  - Hydrops fetalis
  - Intrauterine growth restriction
  - GI
  - Hepatomegaly/hepatitis
- Hematologic:
  - Lymphadenopathy, anemia, thrombocytopenia
- Macrocysternum:
  - Edema, rash (pemphigus syphiliticus), rhagades
- Orthopedic:
  - Osteochondritis, periostitis
  - Wimberger's sign
  - Rhagades of Parrot
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Disclosure

W. Christopher Golden, MD has no relevant relationships with commercial companies to disclose.
Pediatric Grand Rounds - UT Health SA

Syphilitic embryopathy/fetopathy
Rodriguez-Cerdeira C and Silami-Lopes VG, Actas Dermosifilogr. 2012  103 : 679-693


CS in Baltimore, 1996-1997
- CS in Baltimore increased nearly five fold between 1993 and 1996
- 15 month cohort (1/96-3/97)
  - Primary characteristics: single, AA, unemployed, drug abuse (heroin and cocaine)
  - Key differences: Cases had late (third trimester) diagnosis/prenatal care
    - 80% with missed opportunity for testing (SS/incarceration)

https://www.cdc.gov/std/stats16/figures/44.htm

Courtesy of Dr. Cesar V. Peña, Center for Sexually Transmitted Infection Prevention, Maryland DHMH
• Retrospective analysis of ~ 1600 women delivering at JHH in 2012 with at least 1 prenatal visit (or hospital visit in pregnancy)
  • 28.4% had an HIV retest (1st and 3rd trimester test)
  • 78.7% of women had both an intake and a 3rd trimester syphilis test, with a statistically lower rate in women without repeat HIV testing (89.5%).

### Syphilis: Diagnosis

- **Physical exam findings (noted)**
- **Maternal history**
  - Non-treponemal tests
    - IgM/IgG antibodies to lipoidal material from damaged cells as well as lipoprotein-like material and cardiolipin from treponemes
    - Appear between 3-6 weeks after infection
  - Quantitative non-treponemal tests used to establish baseline of reactivity, recovery after treatment, evidence of relapse/reinfection

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<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<tbody>
<tr>
<td></td>
<td>Primary</td>
<td>Secondary</td>
</tr>
<tr>
<td>VDRL</td>
<td>98 (94-98)</td>
<td>98 (85-100)</td>
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<tr>
<td>RPR</td>
<td>94 (90-98)</td>
<td>98 (90-98)</td>
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<tr>
<td>TRUST</td>
<td>85 (70-86)</td>
<td>98 (99-99)</td>
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Adapted from Seka, M. et al., AM J Obstet Gynecol 2009 (42): 1090-1095
Syphilis: Diagnosis

- Early treponemal tests
  - IgM/IgG antibodies from reactive serum bind to fixed *T. pallidum*
  - FTA-ABS, MHA-TP, TPPA, THPA
  - Used as confirmation of non-treponemal results.
  - Remain positive after initial infection

<table>
<thead>
<tr>
<th>Sensitivity during stage of infection, % (range)</th>
<th>Specificity, % (range)</th>
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<tbody>
<tr>
<td>Preval</td>
<td>Secondary</td>
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<tr>
<td>MHA-TP</td>
<td>76 (69-90)</td>
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<tr>
<td>TPPA</td>
<td>98 (86-100)</td>
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<tr>
<td>TPHA</td>
<td>86</td>
</tr>
<tr>
<td>FTA-ABS</td>
<td>84 (70-100)</td>
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Adapted from Seña AC, et. al., Clin Infect Dis 2010 51: 700-708

The New Wave of Treponemal Tests

- **Rapid Tests:** Measure IgM, IgA, IgG against recombinant *T. pallidum* antigens with immunochromatographic strips. Cheap ($1-3/kits), rapid to result (5-20 minutes).
  - Sensitivity 95-98%, specificity 93-96%

- **Chemiluminescence immunoassays (CIA):** Paramagnetic microparticles coated with recombinant antigens, measure IgG/IgM antibodies.
  - Sensitivity 95-98%, specificity 99%
  - 95-97% sensitivity for primary syphilis
  - 98% sensitivity for untreated syphilis

- **Enzyme Immunoassays (EIA):** Measure IgG or IgG/IgM against wild-type or recombinant *T. pallidum* antigens
  - Approved for clinical diagnostic use by FDA in 2001.
  - Sensitivity 85-99%, specificity 98-100%

Note: CIA/EIA become positive at 14-21 days after infection

Reverse Sequence (RS) Syphilis Screening

Non Treponemal Serologic Test
(i.e., RPR)

Treponemal Serologic Test
[Confirmation] (i.e., FTA-ABS, TP-PA)

Treponemal Specific Serologic Test
(i.e., CIA/EIA)

Non-Treponemal Serologic Test
(i.e., RPR)

Rationale:
- CIAs/EIAs are highly automated
- Eliminates manual pipetting and human subjectivity
- Allows for rapid processing of high-sample volumes

Discordant Results: RS Syphilis Screening

- 56% of patients with positive EIA screens had negative RPR
- EIA "red flagged" 3% of patients who would not have been detected otherwise
- Differences in the way each of the 4 labs handled results
  - Some performed second treponemal test on EIA+/RPR- samples

At that time, CDC recommended:
- No action in patients with history of syphilis with treatment
- Second treponemal test in patients without history of syphilis treatment
- If test positive, treat patient to prevent complications from untreated syphilis

MMWR, 2008 57: 872-875

Discordant Results: RS Syphilis Screening

- Percentage of EIA/CIA reactive sera was greater in the high prevalence population
- Percentage of discordant syphilis tests (EIA/CIA reactive, RPR non-reactive) was greater in the low prevalence population
- Among the discordants, a greater percentage of FTA-ABS or TP-PA non-reactive tests were seen in the low prevalence population

Adapted from MMWR, 2011 60: 133-137

Consensus

- Use a second treponemal assay (preferably TP-PA) to settle discrepant syphilis testing
- Consider data other than the results of serologic tests
- Continue to use the traditional non-treponemal (RPR) test as the first screen

MMWR, 2011 60: 133-137
The “Costs” of Syphilis Testing
Mishra, et al. (Toronto)
Sex Transm Dis 38: 190-196 (2011)
• 3-fold increase in confirmed cases after switch from RPR to EIA screening.
• Increased testing at community level
Chuck, et al. (Edmonton)
• $461 CAN incremental cost-effectiveness ratio (ICER) savings per correct diagnosis overall with EIA/RPR/IL testing (relative to traditional testing)
• $1358 CAN ICER increase per correct diagnosis in the prenatal population
Owusu-Edusei, et al. (CDC)
Sex Trans Dis 38: 1-7 (2011)
• $50 greater ICER increase for RS testing (vs traditional testing)
• 118 additional confirmed cases
• Roughly 3-fold increase in follow-up, 900 over treated patients
Binnicker, et al. (Rochester, MN)
• Cost of reagents to perform treponemal screening assays range from $3-$19 US per patient. Cost for RPR: $0.51
Binnicker, et al.
• In low prevalence population, RS testing identified two patients with negative RPR, 6 false positives

What Does a Positive EIA/CIA Test Mean?
CIA+/RPR-/TP-PA+ pts.
Male HIV+
Homosexual
Prior Hx syphilis
Higher Median CIA index values

RS Testing: The Johns Hopkins Experience
• June, 2011: RS testing began at JHH
  • CIA, then RPR, then FTA-ABS
2016 numbers
  ~37,000 assays annually
  • Roughly 700 assays per week

RS Testing: The Johns Hopkins Experience
• September, 2011: First neonatal controversy
  • 36 week twins, ART pregnancy, parents are married couple from West Africa
  • Denied any recent or past history of syphilis, other treponemal/spirochetal diseases, recent travel
  • Mother was RPR non-reactive at outside clinic, but no prior history of CIA testing.
  • Testing at delivery: CIA+/RPR NR/FTA-ABS+
  • ID consultation: Test infants… both who resulted the same!
  • Delay in testing results
  • Rx: 10 days of PCN for both babies

What Does a Positive CIA/EIA Test in Pregnancy Mean?
• If a treponemal test (e.g., EIA or CIA) is used for antepartum syphilis screening, all positive EIA/CIA tests should be reflexed to a quantitative nontreponemal test (RPR or VDRL).
• If the nontreponemal test is negative, then the results are considered discrepant and a second treponemal test (TP-PA preferred) should be performed, preferably on the same specimen.
• If the second treponemal test is positive, current or past syphilis infection can be confirmed.
  • For women with a history of adequately treated syphilis who do not have ongoing risk, no further treatment is necessary. However, a history of inadequate treatment requires further evaluation and a determination of possible options.
• If the second treponemal test is negative, the positive CIA/EIA is more likely to represent a false-positive test result in low-risk women with no history of treated syphilis.
  • If the woman is a live risk for syphilis, lack of symptoms of primary syphilis, has a partner with no clinical or serologic evidence of syphilis, and is likely to follow up, repeat serologic testing within 4 weeks can be considered to determine whether the EIA/CIA remains positive or if the RPR/VDRL or the TP-PA becomes positive. If both the RPR and TP-PA remain negative, no further treatment is necessary.
  • If follow-up is not possible, women without a history of treated syphilis should be treated according to the stage of syphilis.

What Does a Positive CIA/EIA Test in Pregnancy Mean?

CIA/EIA RPR FTA-ABS/TPPA Interpretation
N NR NR Mother is not infected with syphilis
E R N R Mother:
  a. is not infected with syphilis (false positive CIA/EIA)
  b. has early syphilis infection (with negative RPR and FTA-ABS or TPPA)
P NR R Mother:
  a. had syphilis (in the past or in this pregnancy) and was treated
  b. has untreated, latent syphilis infection
  c. has untreated, early syphilis infection
P R R Mother:
  a. had syphilis (in the past or in this pregnancy) and was treated
  b. has untreated, latent syphilis infection
  c. has untreated, early syphilis infection

N = negative; E = equivocal; P = positive; NR = non-reactive; R = reactive

RS Testing: The Johns Hopkins Experience

• Fall, 2011: Discovery of systems issues
  • No routine syphilis testing available on weekends!
    • Samples sent late Friday/Saturday/Sunday/holidays batched and run on Monday
    • Significant impact on patient care
    • NB discharges could be delayed
  • No clear management plan for mothers and babies in the RS paradigm available.

RS Testing: The Johns Hopkins Experience

• Fall, 2011: Addressing the issues
  • Representatives from Neonatology, Pediatric and Adult Infectious Diseases, Pathology (Diagnostic Immunology), and Gynecology/Obstetrics collaborated on two key fronts
  • RS testing algorithm
    • Focus on maternal and neonatal testing
  • Syphilis testing on weekends
    • Diagnostic Immunology agreed to dedicate a technician to run CIA/RPR every Saturday and most Sundays
    • Syphilis testing in Newborn Nursery, NICU, and L&D to receive priority

Congenital Syphilis: Diagnosis and Treatment

Proven or highly probable neonatal disease
- Abnormal PE
- Non-treponemal titer 4x+> than mother
- Darkfield +
  LP, long bone films, CBC with platelets, transaminases, ophthalmology
  (JHH-Abdominal sonogram, ABR)
  IV PCN G 100,000-150,000 U/kg/d, 10 days q12 hrs days 1-7, q8 hrs days 8-10
  OR
  IM procaine PCN 50,000 U/kg/day for 10 days
Inadequate maternal treatment (undocumented treatment, treatment <4 weeks before birth, non-PCN based regimen, relapse by rise in non-treponemal titer)
- Normal PE
- Non-treponemal titer <4x maternal
  Nothing
  Clinical/serologic follow-up, and IM Benzathine PCN 50,000 U/kg once+^
Adequate maternal treatment, no relapse
- Normal PE
- Non-treponemal titer <4x maternal
  Nothing
  Clinical/serologic follow-up, and IM Benzathine PCN 50,000 U/kg once+^ some experts would not treat infant; & some experts would give 1 dose of benzathine if follow-up cannot be assured.
Adapted from CDC 2015 Sexually Transmitted Diseases Treatment Guidelines, https://www.cdc.gov/std/tg2015/syphilis-pregnancy.htm

What Does a Positive CIA/EIA Test in Pregnancy Mean?

How do you reconcile discordant maternal testing in neonatal management?
  • Maternal physical exam
  • Maternal history
    • Does/did she ever have syphilis? Was she treated?
    • Prior history of STIs
    • Syphilis Rx in pregnancy
    • Self report
    • Call local health department
  • Does/did she have any other treponemal diseases (i.e., pinta, yaws)? Other spirochetal diseases (Lyme, leptospirosis)?
  • Is she high risk?
    • HIV positive
    • History of sexually transmitted illness (STI) in herself or in partner
    • active illicit drug abuse
    • multiple sexual partners
    • limited or no prenatal care
  • If any of these risk factors are present, be highly suspicious, even with “normal” serologies!
  • Don’t forget to test mother for HIV!
  • Test the infant using the same RS paradigm

RS Testing: The Johns Hopkins Experience

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RS Testing: The Johns Hopkins Experience

Studying the outcomes of our intervention

Did our medical teams follow the algorithm?

How did our algorithm affect in house patient management?
- Overtreatment or undertreatment?

Did we miss any CS babies?

May W. Chen, MD
Neonatology Fellow

Ibukunoluwa C. Akinboyo, MD
Pediatric Infectious Disease Fellow

Chen and Akinboyo, et. al., unpublished data

RS Testing: The Johns Hopkins Experience

- Retrospective review of all women admitted to Labor and Delivery from December, 2011-May, 2014
- Stratified into three groups (based on RS testing):
  - True positives: CIA+/RPR+
  - False Positives: CIA+/RPR-/FTA-ABS-
  - Discordants: CIA+/RPR-/FTA-ABS+
- Evaluated management of neonates in NBN/NICU and reviewed data (when available) on early infant course, with attention to any with subsequent syphilis diagnosis.

Chen and Akinboyo, et. al., unpublished data
Within the true positive and presumed false positive groups, clinical practice corresponded well with the Red Book evaluation/management guidelines and our algorithm. However, in the discordant group, clinical practice fully corresponded with our clinical algorithm in only 23.8% of cases. Within the discordant group, deviation from the algorithm to perform no neonatal testing occurred due to prior maternal yaws infection (n=2), pre-pregnancy maternal syphilis with confirmed treatment (n=9), or maternal IVIG therapy during pregnancy (n=2). As a result of maternal RS testing, 8 infants in the RS discordant group that would not have been tested (based on an initial negative maternal RPR) underwent complete or partial CS evaluation. None of those infants had findings consistent with congenital syphilis. Of the 12 infants in the discordant group (57%) who had 6-month follow-up data available, none had evidence of syphilis.

### Summary

- Syphilis remains a difficult diagnosis in newborns.
- RS testing provides many conveniences to laboratories and hospitals, but its utility must be considered in the context of both high-risk and low risk STI populations.
- Clinicians still must rely on clinical findings in the maternal-child dyad, maternal history and risk factors, and laboratory testing to confirm cases of CS.
- Collaboration between adult and pediatric clinicians across local, state, and federal jurisdictions must continue to improve outcomes in neonates and children at risk for acquisition of CS.

### Thank you!

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