C-REACTIVE PROTEIN (CRP) PLUS NASOPHARYNGEAL/OROPHARYNGEAL AND WHOLE BLOOD PNEUMOCOCCAL PCR DENSITY TO DIFFERENTIATE PNEUMOCOCCAL FROM NON-PNEUMOCOCCAL PNEUMONIA IN CHILDREN IN THE 7-COUNTRY PNEUMONIA ETIOLOGY RESEARCH FOR CHILD HEALTH (PERCH) CASE-CONTROL STUDY

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INTRODUCTION

High pneumococcal PCR density in the naso/oropharynx (NP/OP) and whole blood (WB) is associated with pneumococcal pneumonia [1], but their imperfect specificity limits its use in ascribing etiology. Elevated C-reactive protein (CRP) is associated with bacterial infection but does not distinguish between bacterial etiologies and has imperfect specificity distinguishing bacterial from viral pneumonia [2]. We assessed effects of combining pneumococcal density with CRP on sensitivity and specificity for distinguishing pneumococcal pneumonia from other pneumonia etiologies in the PERCH study.

METHODS

- PERCH is a 7 country case-control study of WHO-defined severe and very severe pneumonia in children 1-59 months of age.

- **Confirmed bacterial pneumonia**: bacteria detected by blood culture or by lung aspirate or pleural fluid culture or PCR, including **confirmed pneumococcal (Spn)** and **other confirmed bacterial pneumonia cases (non-Spn).**

- **RSV:** respiratory syncytial virus detected by PCR in NP/OP or induced sputum, excluding all confirmed bacterial cases; represents likely viral pneumonia

- **High-density Spn**: Spn PCR density in NP/OP > 6.6 $\log_{10}$ copies/mL (> 4.4 if case received prior antibiotics) or whole blood > 2.2 $\log_{10}$ copies/mL (thresholds derived comparing Spn-confirmed cases to PERCH community controls).

- We compared the percent with both elevated CRP and high density Spn in the different case groups.

- An **Optimal linear combination** of density and CRP was obtained using ROC analysis to derive a score that maximizes the Youden Index [3]; Spn-confirmed cases were compared to cases with other confirmed bacterial <u>or</u> RSV (i.e., combined).

- **Sensitivity** and **specificity** were calculated for the different methods of combining CRP with density; cases with other confirmed bacterial or RSV were combined for this analysis.

RESULTS

- The percent with both NP/OP high-density Spn <u>and</u> CRP ≥ 40 mg/L was higher for Spn-confirmed cases (64%) than for cases with other confirmed bacterial (33%) or RSV (7%); among cases with prior antibiotics, the proportion was higher for RSV (19%) (Figures 1 and 3).

- The 'false positivity' of whole blood high-density Spn <u>and</u> CRP ≥ 40 mg/L was lower (other confirmed bacterial cases=3%; RSV <1%) and sensitivity was also lower (50%) **(Figures 1 and 3)**.

- CRP improved specificity over NP/OP high-density alone by 17-37%, but at loss to sensitivity of 12-25%; the addition of CRP to whole blood had only minor improvements to an already highly specific measure (from 97% to 99%) and with minor losses in sensitivity (from 58% to 52%) **(Figure 3)**.

- The method of an optimal linear combination of CRP with density best distinguished case groups, generally by increasing sensitivity while maintaining fairly high specificity **(Figures 2 and 3)**.

- The **highest specificity** measured was for **high density Spn in NP/OP <u>and</u> whole blood (99%)**, with or without CRP.

- The **highest sensitivity** (87%) while still maintaining ≥ 90% specificity was for the **optimal linear combination of NP/OP density <u>and</u> whole blood <u>and</u> CRP** which also maximized the combination of sensitivity and specificity **(Figure 3)**.

- The linear combinations of CRP with density had higher specificity for RSV cases than for non-Spn confirmed bacterial cases **(Figure 2)**.
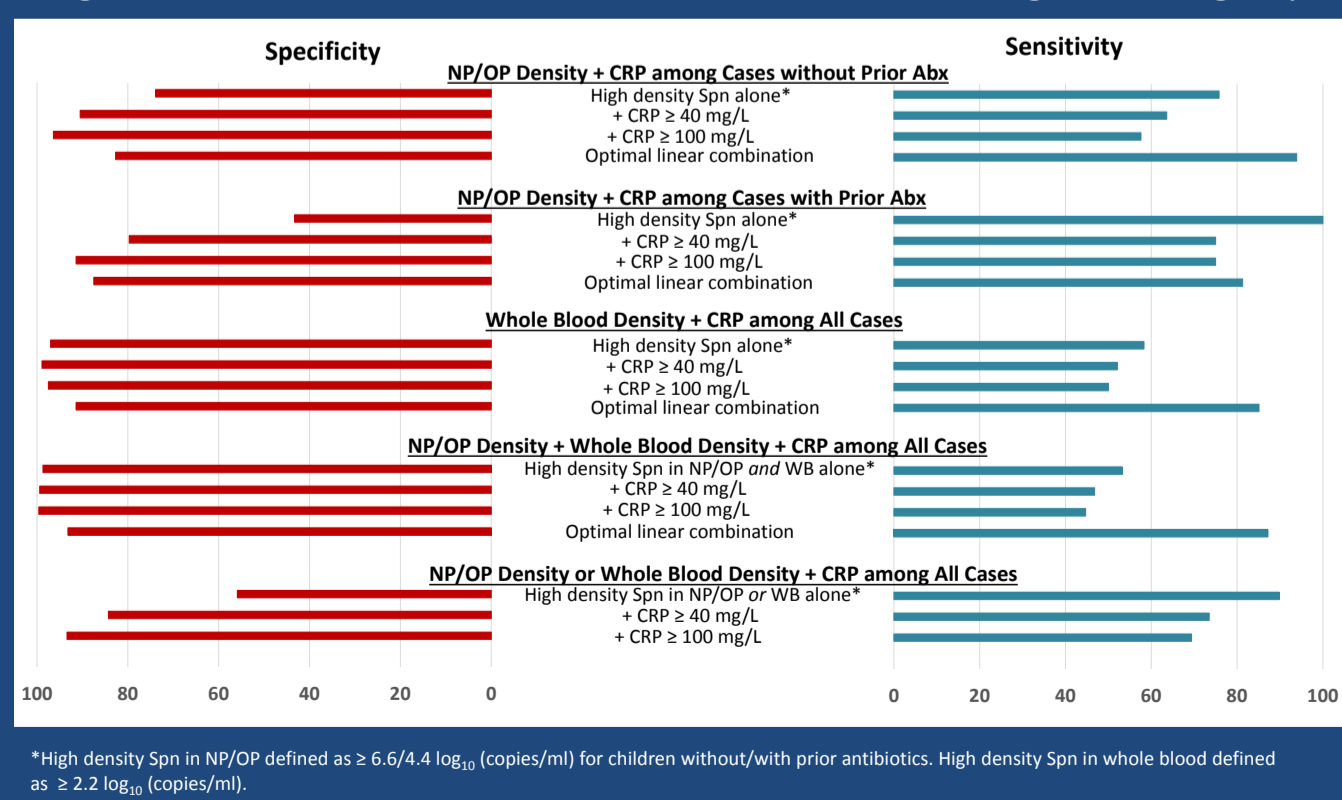
FIGURE 1. NP/OP pneumococcal PCR density and CRP distribution among different case groups by antibiotic use

= area where results expected; **Black %** = % of cases in shaded region; **Red %** = % with high-density pneumococcus in NP/OP or whole blood and CRP ≥ 40 mg/L (red outline)

FIGURE 2. Optimal linear combination of CRP with NP/OP and whole blood pneumococcal PCR density in distinguishing Spn-confirmed pneumonia cases from other confirmed bacterial and RSV cases

NP/OP Density + CRP, Without Prior Antibiotics	NP/OP Density + CRP, With Prior Antibiotics	Whole Blood Density + CRP, With Prior Antibiotics
Positive above line:	Positive above line:	Positive above line:
Spn-confirmed cases: 93.9%	Spn-confirmed cases: 81.3.0%	Spn-confirmed cases: 85.1%
Other confirmed bacterial cases: 57.1%	Other confirmed bacterial cases: 30.0 %	Other confirmed bacterial cases: 37.1 %
RSV cases: 13.4%	RSV cases: 10.5%	RSV cases: 5.6%

● Spn-confirmed cases   ● Other confirmed bacterial cases   ● RSV Cases

CONCLUSIONS

- The method that maximized joint sensitivity and specificity was the **optimal linear combination of NP/OP density <u>and</u> whole blood <u>and</u> CRP** (sensitivity=87%, specificity=93%).

- Having **CRP ≥ 40 <u>in addition to</u> high density Spn in NP/OP** markedly increased specificity over density alone, especially among cases pretreated with antibiotics which reduces density of Spn in the NP/OP (increase in specificity of 35%).

- Sensitivity and specificity of **whole blood high density alone** was similar to that of **CRP ≥ 40 <u>plus</u> NP/OP high density** (difference of 6% in both sensitivity and specificity) among those not pretreated with antibiotics.

- Linear combination 'scores' of CRP and density rather than combining binary cut-offs generally improved sensitivity at little cost to specificity and may be a useful method in etiology analyses.

- One limitation is that co-infection of Spn pneumonia with other pathogens cannot be ruled out, so specificity may not be correct.

- The value of adding CRP will depend on the viral:bacterial case mix (higher with more viral pneumonias, as in the post-PCV and Post-Hib vaccine era), or when high specificity is desired (e.g., estimating vaccine efficacy).

References

1. Albrich WC, Madhi SA, Adrian PV, et al. Use of a rapid test of pneumococcal colonization density to diagnose pneumococcal pneumonia. Clin Infect Dis. 2012;54:601–9
2. Flood RG. The utility of serum C-reactive protein in differentiating bacterial from nonbacterial pneumonia in children: a meta-analysis of 1230 children. Pediatr Infect Dis J. 2008: 27(2): 95.9.
3. Yin J and Tian L. Optimal linear combinations of multiple diagnostic biomarkers based on Youden Index. Stat Med. 2014; 33(8): 1426-40.

Figure 3. Sensitivity and specificity of various definitions for Spn-confirmed cases using other confirmed bacterial cases and RSV+ cases as a single control group
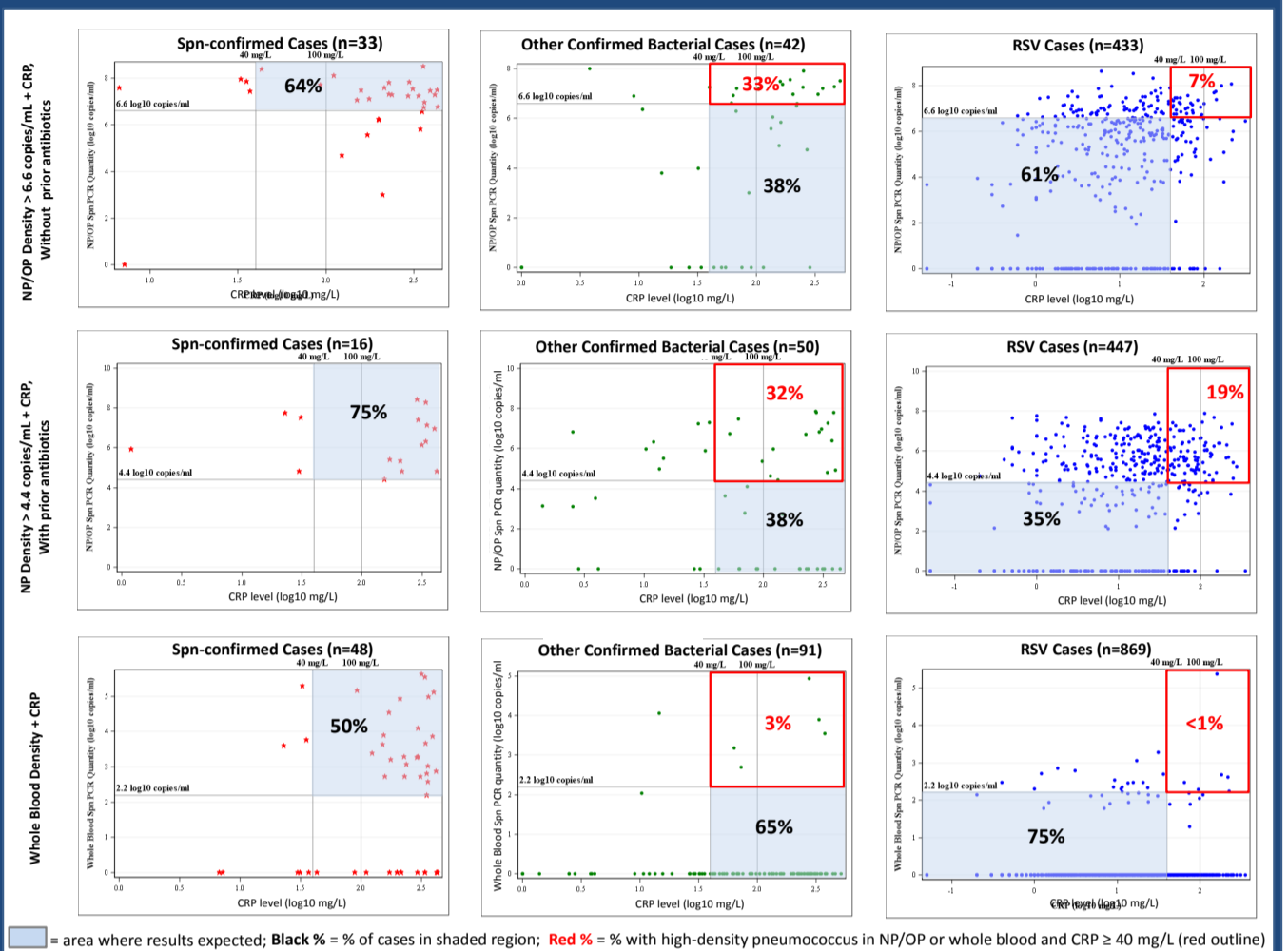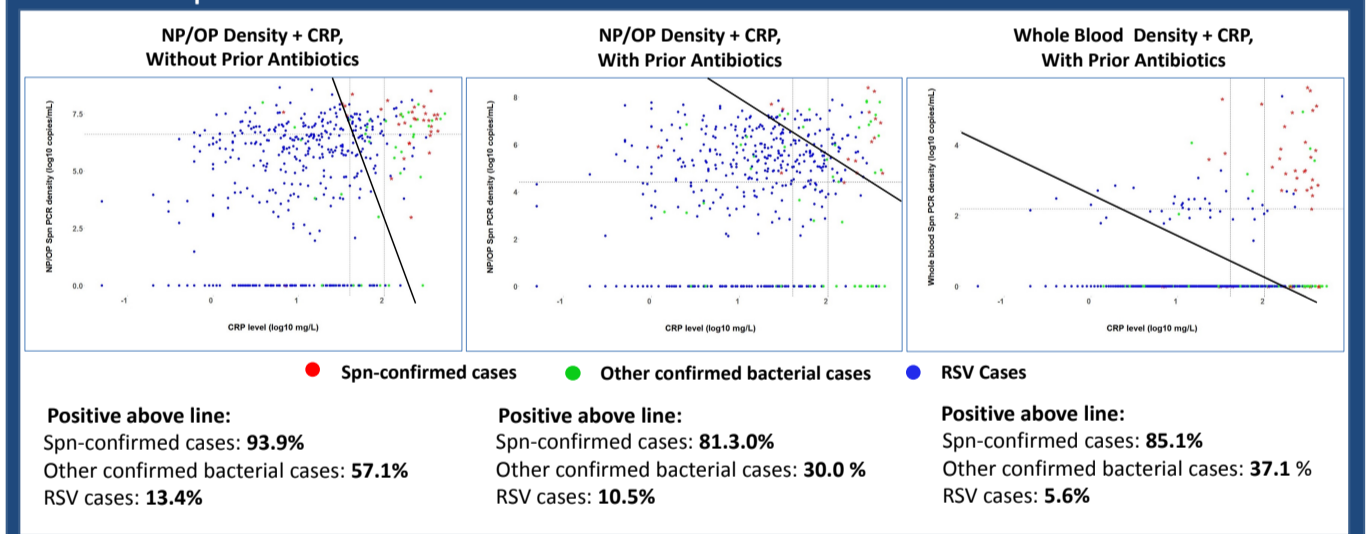

Specificity | Sensitivity

NP/OP Density + CRP among Cases without Prior Abx
High density Spn alone*
+ CRP ≥ 40 mg/L
+ CRP ≥ 100 mg/L
Optimal linear combination

NP/OP Density + CRP among Cases with Prior Abx
High density Spn alone*
+ CRP ≥ 40 mg/L
+ CRP ≥ 100 mg/L
Optimal linear combination

Whole Blood Density + CRP among All Cases
High density Spn alone*
+ CRP ≥ 40 mg/L
+ CRP ≥ 100 mg/L
Optimal linear combination

NP/OP Density + Whole Blood Density + CRP among All Cases
High density Spn in NP/OP and WB alone*
+ CRP ≥ 40 mg/L
+ CRP ≥ 100 mg/L
Optimal linear combination

NP/OP Density or Whole Blood Density + CRP among All Cases
High density Spn in NP/OP or WB alone*
+ CRP ≥ 40 mg/L
+ CRP ≥ 100 mg/L

100 80 60 40 20 0   0 20 40 60 80 100

*High density Spn in NP/OP defined as ≥ 6.6/4.4 $\log_{10}$ (copies/ml) for children without/with prior antibiotics. High density Spn in whole blood defined as ≥ 2.2 $\log_{10}$ (copies/ml).