#499

PILOT EVALUATION OF A QUANTITATIVE SEROTYPE-SPECIFIC URINE ANTIGEN DETECTION (SS-UAD) ASSAY TO IDENTIFY PNEUMOCOCCAL PNEUMONIA IN CHILDREN <5 YEARS

Maria Deloria Knoll for PERCH Study Group[1], and M.W. Pride[2], S. Sebastian[2], B. Hilton[3], R. Isturiz[3], K.U. Jansen[2]

[1]Department of International Health, International Vaccine Access Center, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; Department of Pathology, University of Otago, Christchurch; Microbiology Unit, Canterbury Health Laboratories, Christchurch, New Zealand; Kenya Medical Research Institute-Wellcome Trust Research Programme, Kilifi, Kenya; Medical Research Council Unit, The Gambia; Departments of Pediatrics and Medicine, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland; Centre pour le Développement des Vaccins (CVD-Mali), Bamako, Mali; Boston University School of Public Health, Boston, Massachusetts; University Teaching Hospital, Lusaka, Zambia; Medical Research Council: Respiratory and Meningeal Pathogens Research Unit, University of the Witwatersrand, Johannesburg, South Africa; Department of Science and Technology/National Research Foundation: Vaccine Preventable Diseases Unit; Division of Global Health Protection, Centers for Disease Control and Prevention, Atlanta, Georgia; Global Disease Detection Center, Thailand Ministry of Public Health–US Centers for Disease Control and Prevention Collaboration, Nonthaburi, Thailand; International Centre for Diarrhoeal Disease Research, Bangladesh; Nuffield Department of Clinical Medicine, University of Oxford, United Kingdom; [2]Pfizer Vaccine Research and Development, Pearl River, NY; [3]Pfizer Vaccine World Wide Medical Development & Scientific Affairs

INTRODUCTION

Diagnostic assays to detect pneumococcal pneumonia in children have been shown to have poor sensitivity **and/or** specificity. A Luminex platform-based multiplex serotype-specific urinary antigen detection (SS-UAD) assay has been successfully developed and validated for detecting PCV13-serotype in community acquired pneumonia in adults. A pilot evaluation of the ability of SS-UAD to distinguish carriage from disease in children was conducted in the Pneumonia Etiology Research for Child Health (PERCH) study.

METHODS

PERCH study design: Seven-country case-control study in Africa and Asia [1]

—**Cases**: hospitalized children aged 28 days – 59 months with WHO-defined severe or very severe pneumonia

—**Controls**: age-frequency matched children selected randomly from the community

Pneumococcal testing was performed on urine, blood and naso/oropharyngeal (NP/OP) swabs collected at enrolment from both cases & controls)

- Pneumococcal **colonization** was detected by culture of NP/OP swabs
- Microbiologically confirmed pneumococcal pneumonia (**MCPP**) cases had pneumococcus detected by culture from blood, lung aspirate, or pleural fluid; includes 1 lung aspirate PCR+/culture- case with ST1 detected in induced sputum
- Serotyping was performed by Quellung reaction and/or multiplex PCR

Chest X-rays were interpreted using the WHO method by a trained panel

PCV Coverage in the community: percent with ≥1 PCV dose among all enrolled controls

SS-UAD pilot testing: Eligible sites had ≥1 PCV13-type MCPP case; **eligible cases and controls** (without acute respiratory infection (ARI)) had PCV13-type colonization and ≥500µL urine.

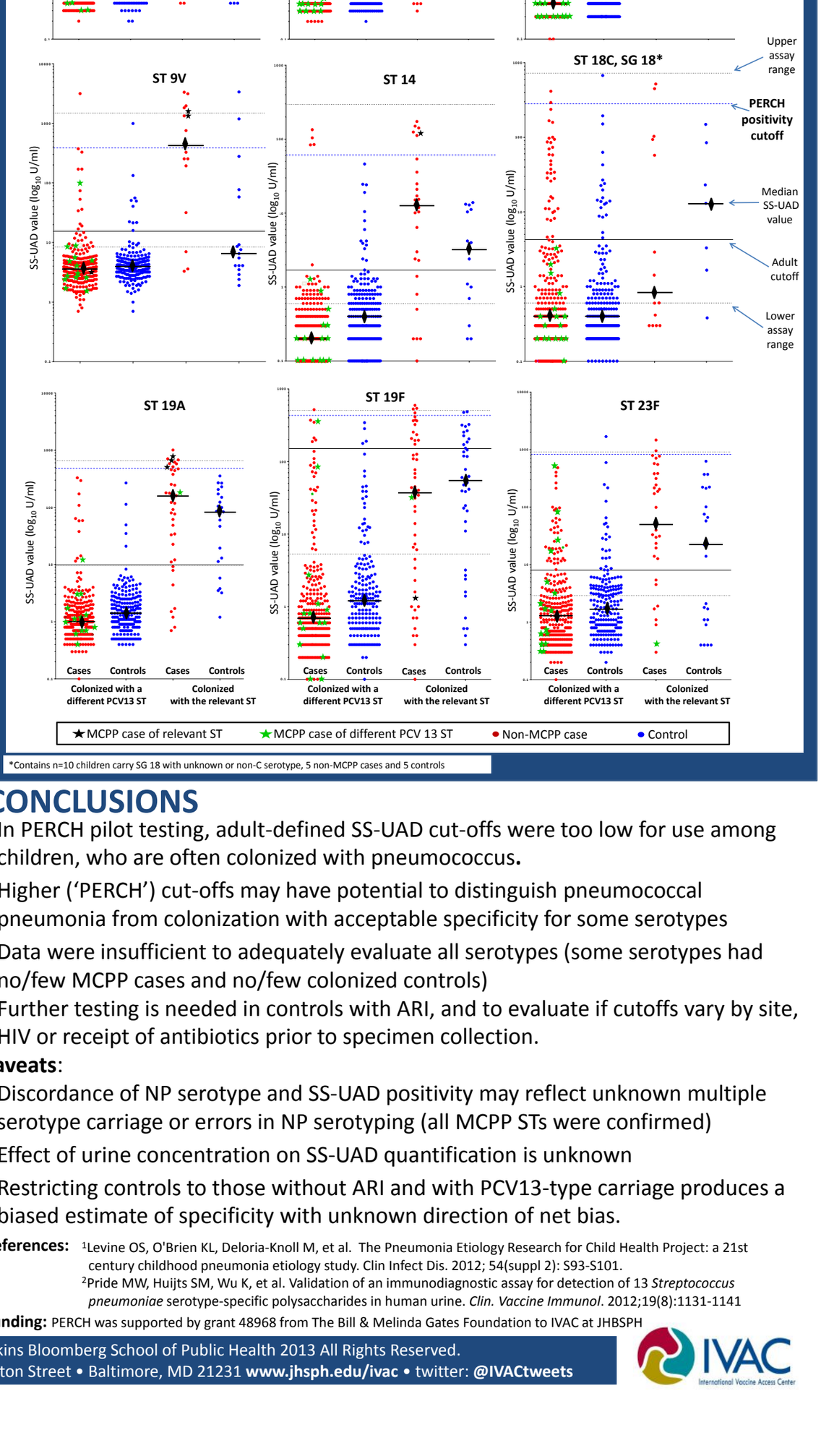
- Urine was tested by SS-UAD at Pfizer Laboratories blinded to case/control and colonization status; urine concentration was not accounted for.
- SS-UAD cannot distinguish within some serogroups: 6A/6C, 7A/7F, 9A/9V and 18A/B/C/F
- PERCH serotype-specific positivity cut-offs were set by Pfizer lab and defined as 'provided at least 95% assurance that ≥96.5% of future negative samples will be below the cut-off'

Analysis: We calculated median SS-UAD value (log_{10} U/ml) and the number and percent above the cut-offs (both the established adult cut-offs used in the CAPiTA Trial [2] and the PERCH cut-offs), by case and control status and by serotype colonization status, for each serotype.

RESULTS

- 4 sites met eligibility criteria for SS-UAD testing: Kenya, The Gambia, Mali, Zambia
- We evaluated n=17 PCV13-type-MCPP cases (representing 8 PCV13 serotypes: 1, 5, 6A, 6B, 9V, 14, 19A, 19F), n=227 controls colonized with a PCV13-type serotype (representing 11 serotypes, 8 of which had at least 10 controls colonized with that serotype), and n=297 non-MCPP cases colonized with a PCV13-type serotype (representing all 13 serotypes).
- 69.4% of PCV13-type colonized controls exceeded adult-defined cut-offs for ≥1 serotype
- **PERCH-defined 'pediatric' cut-offs for SS-UAD 'positivity' (FIGURE)**:
  - Cut-offs for ST3 could not be established (control values exceeded upper limit of assay quantification) *(data not shown)*
  - SS-UAD result was concordant with the invasive serotype of 14/17 (82%) MCPP (★) cases
  - 11/227 (4.8%) control values exceeded cut-offs for serotypes 1, 6A, 9V, 18C, 19F, and 23F (excludes ST3); 8 of the 11 were colonized with the SS-UAD-'positive' serotype.
  - Pilot results suggest cut-offs may distinguish carriage from pneumonia for some serotypes (1, 4, 6B, 14, 19A) but sample size was small, especially for controls carrying STs 1 and 4.
  - 9V had lower specificity (2/15 colonized controls positive); a higher cut-off may be needed
  - Cut-offs may not distinguish carriage from pneumonia well for serotypes 6A and perhaps 19F
  - Carriage in controls was too sparse to evaluate serotypes 5, 7F and 18C
  - Percent SS-UAD-positive increased as specificity of the case definition increased (**TABLE**)
- Controls colonized with the relevant serotype had higher **median SS-UAD** than controls colonized with another PCV13-serotype; comparison to colonized cases varied by ST (**FIGURE**)
- SS-UAD correlated poorly with NP/OP PCR density (Pearson Corr. Coef. ≤0.5 for all serotypes)

FIGURE. SS-UAD results with positivity cut-offs indicated for each serotype, by colonization status and case/control status



*Contains n=10 children carry SG 18 with unknown or non-C serotype, 5 non-MCPP cases and 5 controls

TABLE. SS-UAD positivity in pneumonia cases and controls <5y among those carrying* the relevant PCV13-type serotype, by PCV coverage (excluding ST3 results) *Invasive ST in MCPP cases

Case/Control Definition in order of increasing specificity within Case/Control Group		All Sites			PCV Coverage in the community[2]: High (91.5%) Kenya + Gambia			Med (67.5%) Mali			Low (11.6%) Zambia		
		Total	PERCH Cut-off		Total	PERCH Cut-off		Total	PERCH Cut-off		Total	PERCH Cut-off	
Group	Definition	N[3]	n	%	N[3]	n	%	N[3]	n	%	N[3]	n	%
All Cases	Probable non-Spn Cases[1]	104	10	9.6	63	6	9.5	25	4	16.0	16	0	0.0
	All	308	48	15.6	165	24	14.5	101	21	20.8	42	3	7.1
	CXR+	144	32	22.2	83	20	24.1	37	9	24.3	24	3	12.5
	CXR+ with consolidation	92	25	27.2	39	13	33.3	24	9	37.5	29	3	10.3
MCPP (Spn Conf. Cases)	All	17	14	82.4	6	5	83.3	7	7	100.0	4	2	50.0
	CXR+	14	11	78.6	6	5	83.3	4	4	100.0	4	2	50.0
	CXR+ with consolidation	12	10	83.3	5	4	80.0	4	4	100.0	3	2	66.7
Non-MCPP Cases (no conf. Spn)	Probable non-Spn Cases[1]	104	10	9.6	63	6	9.5	25	4	16.0	16	0	0.0
	All	293	37	12.6	161	21	13.0	94	14	14.9	38	2	5.3
	CXR+	130	21	16.2	77	15	19.5	33	5	15.2	20	1	5.0
	CXR+ with consolidation	70	15	21.4	34	9	26.5	20	5	25.0	16	1	6.3
Controls	Without ARI[4]	216	8	3.7	131	6	4.6	34	0	0.0	51	2	3.9

[1]Non-pneumococcal organism isolated from normally sterile site, high density (>5.9 log_{10} copies/mL) NP/OP PCR *H. influenzae*, or RSV or *B. pertussis* detected in NP/OP or induced sputum by PCR. Excludes MCPP cases and cases with high density pneumococcal PCR in NP/OP (>6.9 log_{10} copies/mL) or whole blood (>2.2 log_{10} copies/mL).
[2]Kenya uses PCV10; other sites use PCV13. [3]Total N includes some children twice because they had more than one ST detected in NP/OP. [4]ARI: Acute respiratory Illness

CONCLUSIONS

- In PERCH pilot testing, adult-defined SS-UAD cut-offs were too low for use among children, who are often colonized with pneumococcus**.**
- Higher ('PERCH') cut-offs may have potential to distinguish pneumococcal pneumonia from colonization with acceptable specificity for some serotypes
- Data were insufficient to adequately evaluate all serotypes (some serotypes had no/few MCPP cases and no/few colonized controls)
- Further testing is needed in controls with ARI, and to evaluate if cutoffs vary by site, HIV or receipt of antibiotics prior to specimen collection.

Caveats:

- Discordance of NP serotype and SS-UAD positivity may reflect unknown multiple serotype carriage or errors in NP serotyping (all MCPP STs were confirmed)
- Effect of urine concentration on SS-UAD quantification is unknown
- Restricting controls to those without ARI and with PCV13-type carriage produces a biased estimate of specificity with unknown direction of net bias.

References: [1]Levine OS, O'Brien KL, Deloria-Knoll M, et al. The Pneumonia Etiology Research for Child Health Project: a 21st century childhood pneumonia etiology study. Clin Infect Dis. 2012; 54(suppl 2): S93-S101.
[2]Pride MW, Huijts SM, Wu K, et al. Validation of an immunodiagnostic assay for detection of 13 *Streptococcus pneumoniae* serotype-specific polysaccharides in human urine. *Clin. Vaccine Immunol*. 2012;19(8):1131-1141

Funding: PERCH was supported by grant 48968 from The Bill & Melinda Gates Foundation to IVAC at JHBSPH

PERCH Pneumonia Etiology Research for Child Health

Johns Hopkins University International Vaccine Access Center • 415 N Washington Street • Baltimore, MD 21231 **www.jhsph.edu/ivac** • twitter: **@IVACtweets**

IVAC International Vaccine Access Center