PP5.113 - Predictive Value of Non-Invasive diagnosis of Primary *Pneumocystis* Infection in Infants at Autopsy

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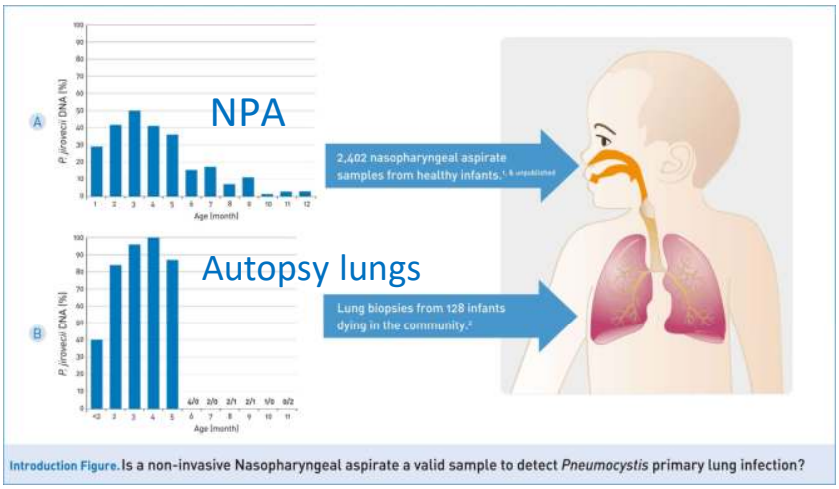
INTRODUCTION

- The primary *Pneumocystis* infection develops before 6 months of age and goes undetected.
- This infection has been associated to a Th2 - type lung environment and to clinical entities like Unexplained Infant Death (SIDS), Respiratory Distress of Pre-term infants, and infant bronchiolitis. Related causality has not been established.
- nPCR of nasopharyngeal aspirate samples (NPA) yield 30-45% at 2 - 5 months of age in healthy infants. However, examination of autopsy lung samples from infants dying in the community give lung-yields of over 90% (Introduction Figure).
- Whether detection of *Pneumocystis* in a NPA predicts lung infection in immunocompetent infants has not been studied because lung biopsies cannot be obtained from healthy infants.



Introduction Figure. Is a non-invasive Nasopharyngeal aspirate a valid sample to detect *Pneumocystis* primary lung infection?

METHODS

- The Ethics Committee of the University of Chile School of Medicine approved the study. All autopsies were legally required.
- *Pneumocystis* was sought in paired NPA and lung biopsy samples from 27 infants (age 3.2 (1.0 to 8.9 months)) dying in the community using *P. jirovecii*-DNA amplification of the mtLSUrRNA by nested-PCR.
- NPA sampling used a sterile, outer surface only lubricated with saline 6 Fr catheter, inserted parallel to the palate until reaching the pharynx. Gentle vacuum pump removal with rotation. Washing contents by suctioning with 7 ml sterile saline. Repeating the procedure in the other nostril.
- For tissue sampling, one lobe (normally the right upper lobe) was collected at autopsy using sterile precautions and taken to a laminar flow hood, where it was weighed and dissected with sterile precautions. Inner tissue was sampled in one, two, or three 0.4 gr aliquots to amount 3% of the lung weight for DNA extraction using QIAmp DNA extraction kit (QIAGEN) and *P. jirovecii*-specific primers for DNA amplification.
- Gene sequencing was attempted in paired samples by re-amplifying mtLSUrRNA with Platinum Taq DNA polymerase and purification of amplicons using the Wizard SV Gel and PCR Clean-up System (Promega). Data quality was controlled using 4Peaks software and nucleotides were aligned using multiple sequence alignment software (SeaView). Nucleotide polymorphisms were determined at previously identified heterozygous positions.

RESULTS

Nasopharyngeal aspirate sample	*Pneumocystis* in the lung		Total
	Positive	Negative	
Positive	*a* = 16	*c* = 4	20
Negative	*b* = 1	*d* = 6	7
Total	17	10	27

Pair	Sample type	Position of polymorphisms in mt26S gene, relative to the reference sequence (M58605).				
		80	**81**	**85**	**88**	**248**
1	NPA	C	C	A	A	ND
	Lung biopsy	C	C	A	A	C
2	NPA	C	C	A	A	C
	Lung biopsy	C	C	A	A	C
3	NPA	ND	ND	ND	ND	C
	Lung biopsy	C	C	T	A	C
Ref. seq. (GenBank number M8605)		*C*	*C*	*C*	*A*	*C*

CONCLUSIONS

- Double-NPA as described has 94% sensitivity, 60% specificity, 80% PPV, 85% NPV, and 81.45% accuracy to detect lung infection in infants at an average age of 3.2 months.
- NPA refining is desirable.
- Genetic homology between paired *Pneumocystis* isolates suggest that the isolates sequenced were not different.
- Validation of NPA will also need to be tested in immunocompetent adults who may carry minimal burdens of *Pneumocystis* in their lungs

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