Investigation of Infectious Etiologies in the Lower Respiratory Tract from Pediatric Patients with Unexpected Cardiopulmonary Deterioration using Next-Generation Sequencing

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Abstract

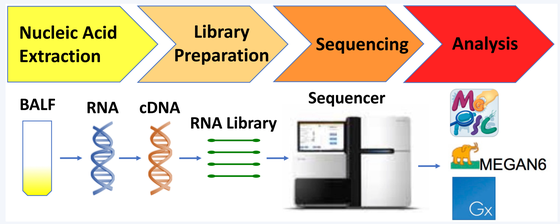
Background : In pediatric patients, unexpected cardiopulmonary deterioration with or without following cardiopulmonary arrest (CPA) are rare events, but can be caused by any of several etiologies, including infectious diseases. The most common cause of out-of-hospital CPA in children ≤12 years old was sudden infant death syndrome (SIDS), whereas infectious diseases were responsible for approximately 10% of the CPA cases. However, the role of infection may have been underestimated as triggers of SIDS or CPA. This study aimed to investigate the infectious etiologies in pediatric patients with unexpected cardiopulmonary deterioration using next-generation sequencing (NGS).

Methods : A total of 16 pediatric patients who were admitted to the pediatric intensive care unit with unexpected cardiopulmonary deterioration with or without following CPA were enrolled. Ten bronchoalveolar fluid (BALF) and six transtracheal aspirates (TTA) samples obtained in the acute phase were used to prepare NGS libraries and were analyzed using metagenome analysis tools.

Results : In ten of 16 patients, one or more bacterial/viral pathogens were detected in the BALF or TTA specimens using NGS. Compared to the conventional culture and viral antigen test results, an additional 6 bacterial (e.g., Chlamydia trachomatis) and 4 viral pathogens (e.g., coxsackievirus A6 and human coronavirus NL63) were identified by NGS in four of ten patients in whom no causative pathogen had been identified by conventional culture and viral antigen tests. Notably, sequencing results allowed us to define genotypes for all of the detected viruses in a single NGS assay per patient.

Conclusions : Our results suggest that viral and bacterial infection are common triggers in unexpected cardiopulmonary deterioration in pediatric patients. NGS has the potential to contribute to the clarification of the etiology of pediatric critical illness.

NGS Workflow

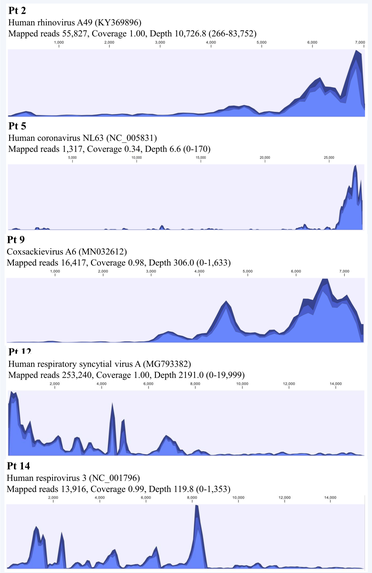


Patient Characteristics & Detected Pathogens (Table 1)

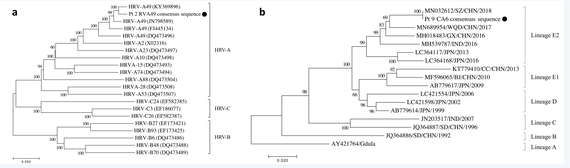
Pt No.	Age	Sex	Underlying disease	Diagnostic category	Sample type	NGS results (RPM)		Conventional test results[d]
						Bacteria	Virus	
1	6y0m	M	-	CPA	BALF	-	-	-
2	9y8m	F	-	Acute cardiac failure Arrhythmia	BALF	*S. pneumoniae* (101)	HRV-A49 (742)	-
3	0y5m	M	-	Acute respiratory failure	BALF	-	-	-
4	0y2m	F	-	Acute respiratory failure	BALF	*A. guillouiae* (99)	-	-
5	1y3m	F	Emanuel syndrome TOF[a]	Acute cardiac failure	BALF	***M. catarrhalis*** (111) ***H. influenzae*** (78)	HCoV-NL63 (79)	***H. influenzae*** (2+) *S. pneumoniae* (1+) ***M. catarrhalis*** (±)
6	0y2m	M	-	Acute cardiac failure	BALF	-	-	-
7	0y5m	M	-	CPA	BALF	***S. pneumoniae*** (16,417)	-	*H. influenzae* (2+) ***S. pneumoniae*** (2+) *α-Streptococcus sp.* (2+)
8	0y6m	M	-	CPA	BALF	***M. catarrhalis*** (230)	-	*S. pneumoniae* (3+) *H. influenzae* (3+) ***M. catarrhalis*** (3+)
9	1y4m	F	West syndrome[b]	CPA	BALF	-	Coxsackievirus A6 (86)	-
10	9y11m	F	-	Fulminant myocarditis	BALF	-	-	-
11	4y7m	M	-	CPA Fulminant myocarditis	TTA	-	-	-
12	1y7m	F	-	Fulminant myocarditis	TTA	***S. pneumoniae*** (6,126) *S. oralis* (1,451)	**HRSV- A** (1,651)	***S. pneumoniae*** (2+) *H. influenzae* (2+) **HRSV**[c]
13	d17	F	-	Acute cardiac failure Pneumonia	TTA	*C. trachomatis* (76)	-	-
14	6y0m	F	Epilepsy[c]	Acute cardiac failure	TTA	***P. aeruginosa*** (1,508)	PIV-3 (243)	*P. fluorescens/putida* (±) ***P. aeruginosa*** (±) *α-Streptococcus sp.* (±)
15	0y3m	M	-	CPA	TTA	***S. oralis*** (1,783) ***S. pneumoniae*** (1,602) ***S. mitis*** (1,058) *R. mucilaginosa* (869) *S. salivarius* (768)	-	***S. pneumoniae*** **(2+)** ***α-Streptococcus sp.*** (2+) *M. catarrhalis* (±)
16	5y4m	F	-	Acute myocarditis	TTA	-	-	-

Abbreviation: BALF, bronchoalveolar lavage fluid; CPA, cardiopulmonary arrest; HCoV-NL63, Human coronavirus NL63; HRSV, Human respiratory syncytial virus; HRV-A49, Human rhinovirus A49; PIV-3, Parainfluenzavirus 3 (Human respirovirus 3); RPM, reads per million; TOF, Tetralogy of Fallot; TTA, transtracheal aspirates. Bold letters indicate identical pathogens between NGS and conventional tests. [a]A patient with Emanuel syndrome and TOF after Rastelli repair. [b]A patient with West syndrome after group B streptococcal meningitis. [c]A patient with epilepsy after acute encephalopathy. [d]All detected pathogens by conventional methods except for human respiratory syncytial virus in patient 12 reflected bacterial culture test results with transtracheal aspirates. [e]Positive for antigen test.

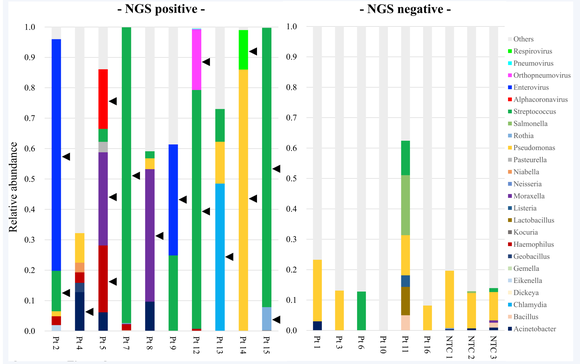
Coverage Plots of Viral Genome (Figure 1)



Phylogenetic Analysis of HRV-A49 & CA6 (Figure 2)



The Composition of Pathogen Reads at the Genus Level (Figure 3)



Summary & Discussion

1. In ten of 16 patients, one or more bacterial/viral pathogens were detected in the BALF or TTA specimens using NGS. Compared to the conventional culture and viral antigen test results, an additional six bacterial and four viral pathogens were identified by NGS (Table 1).
2. Although the presence of a virus had been confirmed in only one patient in conventional tests, NGS detected significant viral sequences in five individuals. Furthermore, NGS permitted us to define genotypes for all of the detected viruses in a single assay per patient (Figure 1 and 2). The CA6 strain detected in patient 9 belongs to lineage E2 and harbors an amino acid change (T283A) in the predicted VP1 protein domain.
3. Although standard methods and positive cutoff values for the detection of pathogens by NGS have not been established, reads of causative pathogens of respiratory infection were dominantly detected (Figure 3).
4. In conclusion, our results suggest viral and bacterial infections may be common triggers of cardiopulmonary deterioration in pediatric patients, and that NGS has the potential to contribute to the clarification of the mechanisms of pediatric critical illness.

Reference

Takeuchi S, Kawada JI, Horiba K, et al. Comprehensive Detection of Candidate Pathogens in the Lower Respiratory Tract of Pediatric Patients With Unexpected Cardiopulmonary Deterioration Using Next-Generation Sequencing. ***Pediatr Crit Care Med*** [published online ahead of print, 2020 Sep 21]. doi: 10.1097/PCC.0000000000002558

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