Respiratory viruses in healthy infants and infants with Cystic Fibrosis: A prospective cohort study.

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Online supplementary material

METHODS

Study design and subjects:

Details on inclusion and exclusion criteria for the study subjects:

In the SCILD study, CF infants were recruited after they had been diagnosed with the Swiss neonatal screening for CF. Infants were enrolled in the study shortly after diagnosis, irrespective of symptoms. In the BILD study, pregnant mothers were recruited at maternity hospitals and practices of obstetricians by advertisements and interviews. Exclusion criteria for the CF cohort were severe comorbidities, exclusion criteria for the healthy cohort were: ethnicity other than white, preterm delivery (<37 weeks), major birth defects, disease or later diagnosis of airway malformation or specific chronic respiratory disease.

Nasal swab procedure:

An anterior nasal swab (FLOQSwabs™, in UTM-RT™ (Copan, Italia)) was collected biweekly by parents in 31 infants with CF and 32 healthy infants after being instructed by study nurses about correct and standardized sampling of the swabs, starting in the 5th week of life. Immediately after acquisition, nasal swabs were sent by mail to our study center and frozen at -80° C, which took a median (IQR) 2 (1-4) days in both study cohorts. Ten different viruses and two atypical bacteria were analyzed in each sample.

Virological analysis:

The following viruses and atypical bacteria were used as outcome parameters in our analysis: Influenza A, Influenza B, RSV, Human Metapneumovirus (hMpV), Adenovirus (ADV), Bocavirus (hBoV), Rhinovirus/Enterovirus (HRV), Parechovirus (hPeV), Coronavirus (hCoV), Parainfluenzavirus (hPIV), *Mycoplasma pneumoniae* (M. pneumoniae) and *Chlamydia pneumoniae* (C. pneumoniae).

Real time PCR using a combination of 7 duplex Respiratory Multi Well System r-geneTM (Influenza A/B, RSV/hMPV, Rhino&EV/CC, ADV/hBoV, HCoV/HPIV, Chla/Myco pneumo

and Parechovirus) commercialized by Argene/bioMérieux (Marcy l'Etoile, France) was performed according to the manufacturer's instructions to analyze the samples. RNA and DNA were extracted from a 400ul sample with the NucliSENS easyMAG (bioMérieux, Marcy l'Etoile, France) and eluted in 110ul.

The real time PCR was performed on different real time PCR machines of Applied Biosystems (7500, 7900HT, QuantStudio® 7 Flex). Sampling quality, extraction and amplification in every sample was evaluated using a HPRT1 cellular gene control (CC) assay using the duplex Rhino&EV/CC r-gene. Samples showing an exponential amplification curve with a CT (crossing threshold) value ≤ 40 were considered as positive. This crossing threshold was applied in all samples, irrespective of viral detection. The PCR tests were done by a certified laboratory which has routinely used the CE marked Respiratory Multi Well System r-geneTM for the detection of respiratory viruses since 2012.

Statistical analysis and sensitivity analyses:

Despite rigid sampling instructions, the number of weeks with missing samples was higher in the group of infants with CF (n=125 vs. n=83). In order to assess whether this could have biased our results, we performed additional statistical analyses. First, we investigated the occurrence of respiratory symptoms in weeks during which nasal swabs were not taken due to e.g. vacation. No difference was found for any kind of respiratory symptoms between healthy infants and infants with CF (OR 0.69, 95% CI 0.17 – 2.75, p=0.6). This was especially true for episodes with respiratory infections, assuring us that missing samples in infants with CF were not due to e.g. hospitalization or infections.

A sample size of 25 children in each group provided a 90% power to detect differences in viral detection, at a two sided 5% significance level, given comparable distribution as shown before (1).

REFERENCES

1. Dijkema JS, van Ewijk BE, Wilbrink B, Wolfs TF, Kimpen JL, van der Ent CK. Frequency and Duration of Rhinovirus Infections in Children with Cystic Fibrosis and Healthy Controls: A Longitudinal Cohort Study. *Pediatr Infect Dis J* 2016; 35: 379-383.

Tables

Table E1: Sensitivity analysis for difference in viral detection in healthy infants and infants with CF

		CF	healthy	Adjusted model		
		N (%)	N (%)	OR	95% CI	*p*
Symptomatic viral detection						
1.	CT 45	96 (17)	163 (23)	0.65	0.44 – 0.96	0.032
2.	CT 35	89 (17)	160 (24)	0.62	0.43 – 0.91	0.015
3.	CT 40+	108 (18)	164 (22)	0.73	0.50 – 1.07	0.1
4.	CT 40b	90 (17)	163 (23)	0.64	0.43 – 0.96	0.03
HRV detection						
1.	CT 45	84 (15)	145 (20)	0.6	0.40 – 0.91	0.016
2.	CT 35	82 (16)	138 (21)	0.64	0.43 – 0.95	0.026
3.	CT 40+	94 (16)	145 (20)	0.63	0.41 – 0.98	0.04
4.	CT 40b	80 (15)	143 (20)	0.63	0.42 – 0.95	0.028
Symptomatic HRV detection						
1.	CT 45	39 (7)	78 (11)	0.51	0.30 – 0.89	0.017
2.	CT 35	38 (7)	76 (11)	0.52	0.30 – 0.9	0.02
3.	CT 40+	39 (7)	78 (11)	0.49	0.28 – 0.85	0.011
4.	CT 40b	38 (7)	78 (11)	0.54	0.31 – 0.93	0.028

Adjusted OR (season and age) for viral detection and symptomatic viral detection comparing healthy and CF infants from multilevel logistic regression in 4 different

models:

1. CT cut off value of 45
2. CT cut off value of 35
3. CT cut off value of 40, but including all samples positive for virus irrespective of CT value
4. CT cut off value of 40, but excluding 3 infants with CF with more than 50% of low quality samples.

In sensitivity analysis 1,2 and 4 the CT thresholds refer to sample quality using the HPRT1 cell gene control and not the detection thresholds for respiratory viruses. For HRV analysis samples with co-detection of other viruses were excluded.

baseline= healthy infants, OR = Odds Ratio, CI= Confidence Interval, p = p-value

total number of samples N(%)