Advances in diagnostics of respiratory viruses and insight in clinical implications of rhinovirus infections

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CHAPTER 5.2

Rhinovirus detection in the nasopharynx of children undergoing cardiac surgery is not associated with longer PICU length of stay; results of the RISK study

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ABSTRACT

Objectives: To determine whether children with asymptomatic carriage of rhinovirus in the nasopharynx before elective cardiac surgery have an increased risk of prolonged pediatric intensive care (PICU) length of stay.

Study Design: Prospective, single-center, blinded observational cohort study.

Setting: PICU in a tertiary hospital in the Netherlands.

Patients: Children under 12 years of age undergoing elective cardiac surgery were enrolled in the study after informed consent of the parents/guardians.

Interventions: The parents/guardians filled out a questionnaire regarding respiratory symptoms. On the day of the operation a nasopharyngeal swab was obtained. Clinical data was collected during PICU admission, and PICU/hospital length of stay were reported. If a patient was still intubated 3 days after operation an additional nasopharyngeal swab was collected. Nasopharyngeal swabs were tested for rhinovirus and other respiratory viruses with PCR.

Measurements and Main Outcomes: Of the 163 included children, 74 (45%) tested rhinovirus positive. Rhinovirus positive patients did not have a prolonged PICU LOS (median 2 days each, p=0.104). Rhinovirus positive patients had a significantly shorter median hospital length of stay compared to rhinovirus negative patients (8 versus 9 days, respectively, p=0.002).

Overall, 97 (60%) of the patients tested positive for one or more respiratory virus. Virus positive patients had significantly shorter PICU and hospital length of stay, ventilatory support, and non-mechanical ventilation. Virus negative patients had respiratory symptoms suspected for a respiratory infection more often.

In 31% of the children the parents reported mild upper respiratory complaints a day prior the cardiac surgery, this was associated with post extubation stridor, but no other clinical outcome measures.

Conclusions: Preoperative rhinovirus PCR positivity is not associated with prolonged PICU LOS. Our findings do not support the use of routine PCR testing for respiratory viruses in asymptomatic children admitted for elective cardiac surgery.

Trial registration: ClinicalTrials.gov Identifier NCT02438293; registration date 5 May 2015.

INTRODUCTION

Symptomatic respiratory infections have been shown to increase the duration of mechanical ventilation, intensive care and hospital length of stay (LOS), and increase the risk of postoperative complications in children following cardiac surgery[1-5]. Previous reports have mainly focused on respiratory syncytial virus (RSV)[1,5-11], but rhinoviruses (RV) may also impact postoperative outcomes[2-4,12-14]. Rhinoviruses in humans worldwide cause more than 50% of upper respiratory tract infections (URTI), such as common cold[12,13,15,16]. They are the leading cause of viral bronchiolitis in infants, the most common virus associated with wheezing in infants[17], prolonged shedding in specific patient groups, and can cause major morbidity and mortality[15,18,19].

Current anesthetic recommendations suggest that children with mild viral respiratory tract infections can safely be operated, but in children with wheezing, purulent secretions, fever, and altered general condition, surgery is recommend to be postponed[20]. Although RV infections, both symptomatic and asymptomatic, are very prevalent, there are no clear markers to help decide to postpone surgery as the evidence is scarce and based on small retrospective perioperative studies with variable symptomatology or outdated diagnostic tests[1,3,20,21]. Children with congenital heart diseases often have chronic and mild upper respiratory tract symptoms that may disappear after surgery. Postponing the operation is not in the best interest of these patients and might also result in empty operating rooms, leading to increased medical costs and waiting lists. Therefore, more evidence is needed to better ascertain which patients are at risk of perioperative complications and a protracted postoperative course.

We designed a single center prospective cohort study to determine whether asymptomatic children, clinically cleared for elective cardiac surgery, who test PCR positive for RV preoperatively, have an increased risk of a prolonged postoperative pediatric intensive care (PICU) LOS compared to those who test negative[22]. We hypothesized that RV positive children would have a prolonged postoperative PICU LOS.

MATERIALS AND METHODS

Design

A detailed RISK study protocol is previously published[22]. This prospective, single-center, blinded observational cohort study was designed to determine the association of RV with PICU LOS in children <12 years undergoing elective cardiac surgery in the Netherlands. Secondary endpoints were hospital LOS, duration of mechanical ventilation (MV), oxygenation index on admission, clinical suspicion of infection post-surgery, and development of adverse events. We also analyzed RV PCR quantification cycle (Cq)-values, RV genotypes, a parental questionnaire, and the occurrence of abnormal findings during intubation with the primary and secondary outcome measures.

Excluded were children admitted to hospital prior to surgery, who required emergency surgery, were not admitted to the PICU after operation (negligible amount), would have a planned prolonged PICU stay (e.g. duct-dependent lesions requiring prolonged prostaglandin infusion), or the lack of informed consent.

Study procedure

The parents/guardians of the eligible children received the information folder and a questionnaire (see supplement 1). The questionnaire consisted of questions regarding respiratory symptoms during the six weeks prior to surgery (e.g. fever, runny nose, coughing, wheezing, etc.), underlying pulmonary disease (of the children and their family), medication use, prematurity, and passive smoking.

As per local protocol, children were admitted one day before the operation (day 0), and clinically assessed and cleared for the operation by the anesthesiologist, cardiologist and cardiac surgeon when no signs of active infection other than rhinorrhea or nasal congestion were present. Written informed consent was then asked by the independent researchers. On the day of surgery (day 1) a nasopharyngeal swab for viral testing was obtained at the induction of anesthesia. Anesthesia was induced with propofol or sevoflurane and maintained with propofol and either sufentanil or remifentanil at the discretion of the anesthesiologist. The anesthesiologist filled out a study form (see supplement 2) detailing findings at direct laryngoscopy (secretions, redness, pus) and other details regarding the induction, the use of steroids, type of anesthesia, and operation conditions. Also, cardiopulmonary bypass times, type of operation, and the Risk Adjustment for Congenital Heart Surgery Score (RACHS) score were collected [23]. After the operation, children were admitted to the PICU, and clinical and laboratory data (blood gas analysis, inflammatory markers, inotrope dose, respiratory conditions, medication and infection) were prospectively collected until PICU discharge, and date of hospital discharge. In case of prolonged PICU admission, a follow-up swab was taken at day 4 of the patients with respiratory support.

All children received 24 hours of peri-operative cefazolin prophylaxis. In the case of postoperative open chest management, cefazolin was switched to flucloxacillin after 24 hours and continued until 24 hours after delayed chest closure. Children were weaned from MV at the discretion of the treating pediatric intensivist.

Definitions

A ‘positive’ questionnaire was defined as any respiratory symptoms in the six weeks prior to surgery, as reported by the parents. Respiratory ‘complaints on admission’ were defined as rhinorrhea and/or nasal congestion present as reported by parents. Hospital LOS was the LOS in the hospital from the day of admission prior to operation until discharge. An ‘adverse event’ included reintubation, readmission, post-extubation stridor, suspected clinical infection, cardiac arrest requiring resuscitation, or arrhythmia requiring treatment. Reintubation was defined as intubation within 48 hours of extubation and ‘readmission’ as readmission within 48 hours of PICU discharge. Post-extubation stridor was defined as stridor within 48 hours of extubation requiring treatment with inhaled steroids, inhaled adrenaline, or systemic steroids. Clinical suspicion of infection was defined as clinical symptoms leading to microbiologic testing and/or antibiotic treatment at the discretion of the treating intensivist. We defined ‘abnormal laryngoscopy’ as redness and/or (purulent) secretions of the larynx, identified by direct laryngoscopy at the time of intubation. No indirect (fiberoptic) laryngoscopy was performed. Chest x-ray on PICU admission was considered abnormal if an

atelectasis and/or a consolidation was present. Non-invasive respiratory support was defined as nasal or mask with continuous positive airway pressure, mask ventilation, or high-flow nasal cannula.

Respiratory virus testing

After publication, our protocol was amended, and all viral respiratory pathogens in our assay were tested[22]. The nasopharyngeal swabs (day 1) were tested for respiratory viruses by means of in-house PCR [24] targeting adenovirus, bocavirus, RV, influenza A/B, RSV, metapneumovirus, para-influenza 1-4, human coronaviruses OC43, HKU1, NL63, and 229E. The day four samples were stored at -80°C, and tested retrospectively. PCR results were blinded for the clinicians and (research) nurses. Genotyping of RV was initially performed by PCR amplification and Sanger sequencing of the VP4/2 region as previously described by Zlateva et al.[25]. Later, bulk sequencing of the same amplicons was performed by next-generation sequencing (NovaSeq6000, Illumina, San Diego, CA, USA). Sequence reads were assembled using SPAdes, version 3.11.1[26]. The reconstructed genome fragments were blasted (BLAST version 2.2.31), against a database of complete genomes of Picornaviridae (database version as of 25 October 2019, prepared with HAYGENS tool, https:// veb.lumc.nl/HAYGENS). For blasting, contigs with a length ranged from 600 to 700 nt concordant to the genome region of rhinoviruses consisting of VP4/2 genes only were used. Both nucleotide and amino acid searches for these regions and scaffolds were performed.

Statistical Analysis

Sample size was initially calculated based on the estimated percentage of RV positive children (20%) and a difference in PICU LOS of 2 days, to be approximately 250 children (ratio 1:4) [22]. However, 11 months after initiation, the percentage of RV positive children turned out to be nearly 50%, therefore the sample size was adjusted to 162 (ratio 1:1) and the protocol was amended accordingly. All continuous data were tested for distribution using the Kolmogorov-Smirnov test. Normally distributed data were presented as means with standard deviations and not-normally distributed data as medians with interquartile range (IQR).

Significant differences between the different groups for the study endpoints were tested with Mann-Whitney U test, t-test, Chi-square or Fisher's exact test where appropriate. Multivariate linear regression analysis was used to adjust for potential confounders, and to identify risk factors. To compare the Cq values at the day of operation with Cq values during the PICU stay, a paired t-test was used. Statistical analyses were performed using IBM SPSS Statistics version 25 software. A p-value of <0.05 was considered statistically significant.

Ethical approval

This study was approved by the medical ethics review committee of the Leiden University Medical Center research file NL51483.058.14 (RV-MM-PED-1), protocol number P14.303.

RESULTS

During the study period, June 2015 – June 2018, 814 children (< 18 years) underwent elective cardiac surgery of which 359 children were eligible for inclusion. One hundred and eighty one parents/guardians were asked for informed consent of which 15 refused (8%), leaving 166/356 (46%) to be included in the study (figure 1). The main reason for exclusion was due to inability to ask informed consent (language barrier, admission during weekend, and staffing constraints). An additional two patients were excluded because of expected prolonged PICU stay (one for mechanical ventilation dependency due to hypotonia and one because of planned reoperation). In 164/166 (99%) patients a nasopharyngeal swab was obtained. For one sample the PCR failed, this patient was excluded from further analysis.

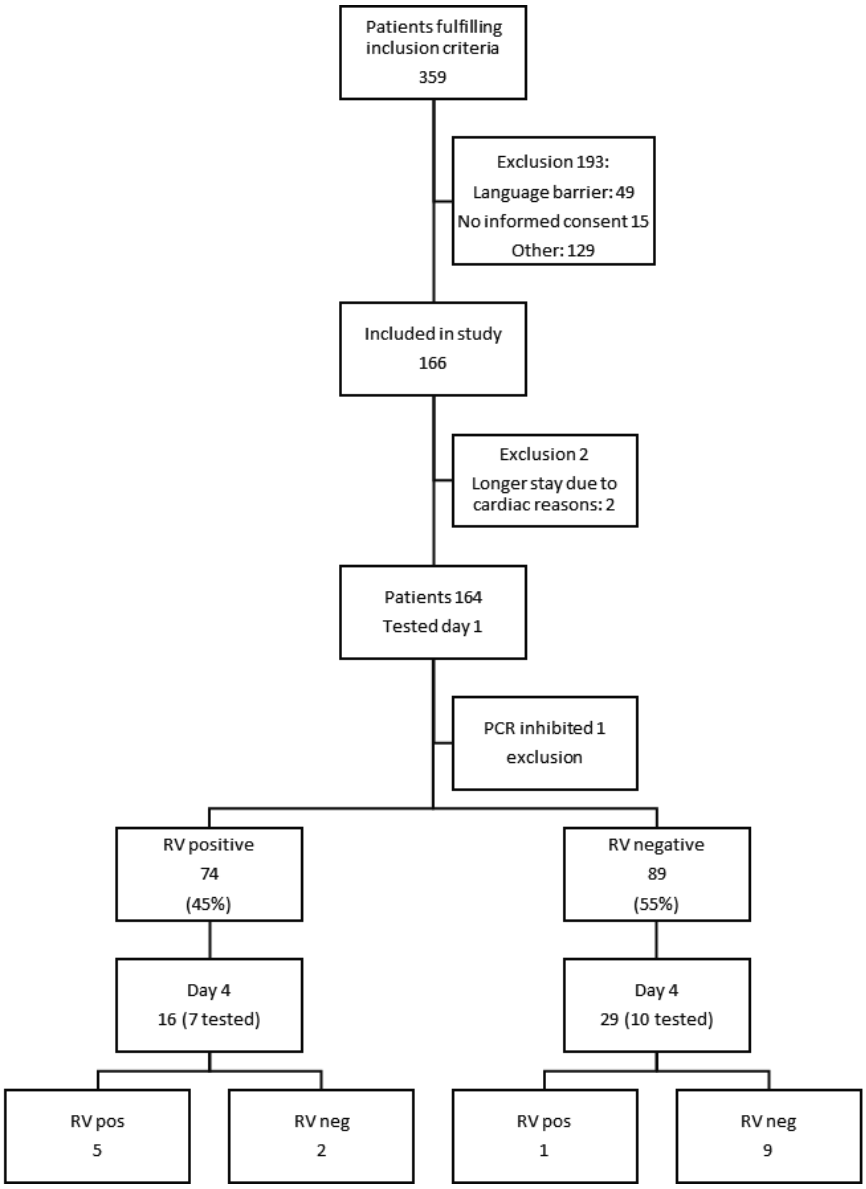

Figure 1. Flowchart of study inclusion.

The median age was 15 months and half of the children were male. Of the 163 children included, 74 (45%) tested RV positive and 89 (55%) RV negative. There were no statistically significant differences in baseline demographics between RV positive and RV negative patients (table 1) with the exception that RV positive patients had more often received steroids during the operation (p=0.026), and a tendency towards current respiratory complaints in the RV positive patients (p=0.070). The most frequent operation indication was biventricular repair (91%). There was no difference in complexity of surgery (RACHS, CPB duration, cross clamp time, delayed sternal closure), anesthetic management (blood products, cumulative fluid) between the groups, or expected mortality (PRISM/PIM) [27,28].

Out of the 74 RV positive patients, 25 (34%) had a coinfection with another virus, of which five patients tested positive for more than two viruses. Of the 89 RV negative patients, 23 tested positive for another virus, of which five patients tested positive for two viruses. After RV, adenovirus was the most prevalent virus (12%), followed by bocavirus (5%). Two patients (1%) were influenza A positive, one influenza B (0.4%) and three patients (2%) tested positive for both rhinovirus and RSV. Respiratory viruses were found throughout the year, with the highest percentage of virus positive patients in June (93%; figure 2).

The outcome variables of RV positive and RV negative patients are listed in table 2. Before and after correction for age (per-protocol) and steroid use during operation, RV positive patients had similar PICU-LOS compared to RV negative patients (median, 2 days each, p=0.104). RV positive patients had a significantly shorter hospital length of stay compared to the RV negative patients (median, 8 versus 9 days, respectively; p=0.002) and were suspected of clinical infection after surgery twice less often than RV negative patients which approached statistical significance (10% versus 21% respectively, p=0.068).

Table 1. Baseline characteristics of included patients.

	all patients n=163	rhinovirus positive n=74	rhinovirus negative n=89	p-value
Demographics				
Age at surgery (months) (median, IQR)	15 (5-47)	12.5 (5.75-44)	16 (4.5-49)	0.987
Male (%)	81 (50)	35 (47)	46 (52)	0.577
Weight at surgery (kg) (median, IQR)	9 (6.3-15.0)	9.1 (6.575-15)	9 (6.1-15)	0.678
Underlying respiratory conditions (%)	22/160 (14)	9/71 (13)	13/89 (15)	0.751
Asthma	4 (25)	0	4 (31)	
Bronchitis	2 (13)	1 (14)	1 (8)	
Tracheomalacia	2 (13)	2 (28)	0	
Pleural fluid	1 (6)	0	1 (8)	
Multiple airway infections	3 (19)	3 (42)	0	
Other	10 (63)	3 (42)	7 (54)	
Infectious respiratory complaints				
Respiratory complaints past 6 weeks (%)	153/160 (96)	69/71 (97)	84 (94)	0.464
Current respiratory complaints (%)	49/160 (31)	27/71 (38)	22 (25)	0.070
Risk assessment				
PRISM score at admission (median, IQR) (II)	6 (3-9)	5 (3-9)	6 (3-9.5)	0.670
PRISM III (median, IQR)	3 (1.75-4)	3 (2-4)	3 (1-4)	0.825
PIM (mean, SD)	-3.68 (0.623)	-3.71(0.598)	-3.66 (0.64)	0.672
Prematurity	19/160 (12)	8/71 (11)	11 (12)	0.832
Passive smoking	12/160 (8)	7/71 (10)	5 (6)	0.312
Operation				0.138
- Univentricular	14 (9)	9 (12)	5 (6)	
- Biventricular	149 (91)	65 (88)	84 (94)	
- CPB duration (minutes) (median, IQR)	91 (59-125)	86 (59.5-124)	97 (59-125)	0.664
- Cross clamp time (minutes) (median, IQR)	57 (31-92)	49 (25-91.25)	64 (33.5-93)	0.251

RACHS score (%)				0.268
1	30 (18)	18 (24)	12 (14)	
2	101 (62)	43 (58)	58 (65)	
3	24 (15)	9 (12)	15 (17)	
4	2 (1)	1 (1)	1 (1)	
Total blood products (median, IQR)	272.5 (200-347.75)	292 (210-395)	260 (180-330)	0.219
Cumulative Fluid (median, IQR)	965 (582-1293)	897 (500.5-1267.5)	977.5 (630.78-1340.75)	0.219
Steroids during operation (%)	45 (27)	27 (36)	18 (20)	0.026
Number of inotropes (%)				0.555
0	5	3	2	
1	53	23	30	
2	52	23	29	
3	4	3	1	
4	1	1	0	
Delayed sternal closure (%)	11 (7)	6 (8)	5 (6)	0.549

IQR: interquartile range, PRISM: pediatric risk of mortality score, PIM: pediatric index of mortality, CPB: cardiopulmonary bypass, RACHS: risk adjustment for congenital heart surgery.

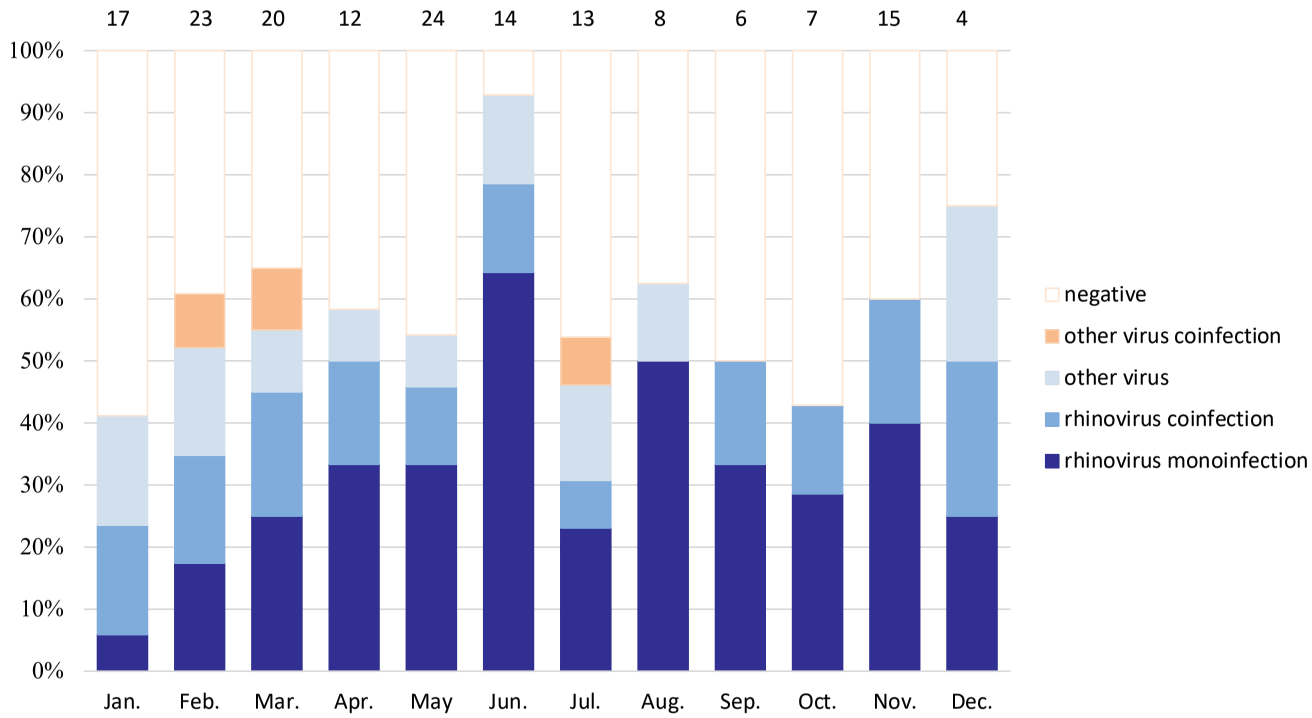

Figure 2. Percentage of rhinovirus mono- and coinfections per month.

Percentages of total number of samples tested per month over the years, total number on top of the bars.

Table 2. Outcome variables of RV-positive versus RV-negative patients.

	all patients n=163	rhinovirus positive n=74	rhinovirus negative n=89	p-value*
PICU LOS (days) (median, IQR)	2 (1-4)	2 (1-3)	2 (1-4)	0.107
Prolonged PICU LOS (=>4 days) (%)	45 (28)	16 (22)	29 (33)	0.094
Hospital LOS (days) (median, IQR)	8 (7-12)	8 (7-10)	9 (7-13.5)	0.002
Duration mechanical ventilation (median, IQR)	0.44 (0.31-1.07)	0.435 (0.2975-0.8350)	0.47 (0.335-1.1)	0.133
Prolonged mechanical ventilation (>48 hours) (%)	22 (13)	11(15)	11 (12)	0.589
Duration of non-invasive respiratory support (hours) (median, IQR)	31 (17-60)	18 (4-52.5)	36 (19.5-61)	0.067
Abnormal laryngoscopy (%)	50(31)	29 (39)	21 (24)	0.064
Oxygenation index on admission to PICU (median, IQR)	2.3 (1.8-3.85)	2.3 (1.8-4.4)	2.4 (1.8-3.7)	0.504
Mean airway pressure (median, IQR)	8.55 (8.0-9.2)	9 (8-9.9)	8.4 (8-9)	0.399
iNO-treatment (%)	2 (1)	0 (0)	1 (2)	0.501
Chest x-ray abnormalities <4 days (%)	54 (33)	25 (34)	29 (33)	0.986
Leucocytes 10x9/L (Median, IQR)	10.1 (7.7-14.2)	11.41 (8.15-14.44)	9.64 (7.3-12.5)	0.050
Extracorporeal life support (%)	3 (2)	1 (1)	2 (2)	0.665
Adverse events (%)	45 (27)	18 (24)	27 (30)	0.531
stridor post extubation (%)	14 (9)	9 (12)	5 (6)	0.138
Suspected of infection postoperatively (%)	26 (16)	7 (10)	19 (21)	0.068
Readmission PICU <48 hours (%)	6 (4)	2 (3)	4 (5)	0.491
Resuscitation (%)	2 (1)	0 (0)	2 (2)	0.501
Rethoracotomy (%)	3 (2)	0 (0)	3 (3)	0.252
JET (%)	4 (3)	3 (4)	1 (1)	0.330
Chylothorax (%)	2 (1)	1 (1)	1 (1)	0.998
AV-block (%)	3 (2)	0 (0)	3 (4)	0.253
Reintubation (%)	2 (1)	0 (0)	2 (2)	0.501
RV Cq value (Mean, SD)		26.8 (4.7)		
Mortality (%)	1(0.6)	0 (0)	1(1)	1.000

PICU: pediatric intensive care unit, LOS: length of stay, IQR: interquartile range, JET: junctional ectopic tachycardia, AV: atrioventricular, RV: rhinovirus, Cq: quantification cycle. * corrected for age and steroid use during operation.

The outcome variables of 'any virus positive' (including RV positive patients, 60%) and 'virus negative' (40%) patients are listed in table 3. The virus negative patients were smaller, younger, had more complicated surgery reflected by their RACHS scores, had more frequent current respiratory complaints, and tended to have more steroids during operation (see supplement 3). After correction for weight, age (per-protocol), and RACHS score, the virus positive patients compared to virus negative patients had significantly shorter PICU LOS (median, 2 versus 3 days, respectively, p=0.048) and hospital LOS (8 versus 9.5 days, respectively, p<0.001). Virus positive patients received shorter ventilatory support (0.41 versus 0.51 days, p=0.042), shorter non-invasive ventilatory support (18 versus 45 hours, p=0.009), and were significantly less often suspected of having a clinical infection postoperatively (10 versus 24%, p=0.017). In virus positive patients, parents reported respiratory complaints on admission significantly more often than in virus negative patients (36 versus 21%, respectively, p=0.03). Similar results, regarding PICU/hospital LOS and duration of mechanical ventilation, were found between virus positive and virus negative patients when we excluded the RV positive patients (data not shown).

Overall, in 50 patients (31%) there was redness or pus during intubation and were suspected of a postoperative clinical infection twice more often than patients without redness or pus (26% versus 12%) (p=0.02). They did, however, not have longer LOS or duration of mechanical ventilation (data not shown).

Table 3. Outcome variables of any virus positive patients.

	any virus positive n=97	virus negative n=66	p-value*
PICU LOS (days) (median, IQR)	2 (1-3)	3 (1-6)	0.048
Prolonged PICU LOS (=>4 days) (%)	20 (24)	25 (38)	0.016
Hospital LOS (days) (median, IQR)	8 (7-10)	9.5 (8-15.25)	<0.001
Duration mechanical ventilation (days) (median, IQR)	0.41 (0.29-0.63)	0.51 (0.35-1.1325)	0.042
Prolonged mechanical ventilation (>48 hours) (%)	11 (11)	11 (17)	0.329
Duration of non-invasive respiratory support (hours) (median, IQR)	18 (5-44.5)	45 (19.88-77.5)	0.009
Abnormal laryngoscopy (%)	35 (36)	15 (23)	0.070
Oxygenation index (Median, IQR)	2.2 (1.8-3.9)	2.6 (1.8-3.7)	0.653
Mean Airway pressure (median, IQR)	8.7 (8-9.5)	8.2 (8-9)	0.292
iNO-treatment (%)	0 (0)	2 (3)	0.162
Chest x-ray abnormalities <4 days (%)	30 (31)	24 (36)	0.606
Leucocytes (median, IQR)	10.6 (8.2-14.3)	9.1 (7.3-13.3)	0.068
Extracorporeal life support (%)	2 (2)	1 (2)	1.000
Adverse events (%)	24 (25)	21 (32)	0.451
stridor post extubation (%)	11 (11)	3 (5)	0.129
Suspected of infection postoperatively (%)	10 (10)	16 (24)	0.017
Readmission PICU <48 hours (%)	4 (4)	2 (3)	0.804
Resuscitation (%)	0 (0)	2 (3)	0.162
Rethoracotomy (%)	0 (0)	3 (5)	0.065
JET (%)	3 (3)	1 (2)	0.648
Chylothorax (%)	1 (1)	1 (2)	1.000
AV-block (%)	1 (1)	2 (3)	0.569
Reintubation (%)	1 (1)	1 (2)	1.000
RV Cq value (mean, SD)	27.9 (5.3)		
Mortality	0 (0)	1 (1.5)	0.405

PICU: pediatric intensive care unit, LOS: Length of Stay, IQR: interquartile range, JET: junctional ectopic tachycardia. AV, atrioventricular, RV: rhinovirus, Cq-value: quantification cycle, *corrected for age, weight and RACHS score

A 'positive' questionnaire, any respiratory symptom in the past six weeks, was found in 96% (153) of the 160 patients (data from 3 children could not be collected). Respiratory symptoms in the past six weeks were not associated with prolonged PICU LOS or any of the secondary outcome measures. Of the 49 (31%) patients whose parents reported mild respiratory complaints on admission, 35 (71%) tested positive for respiratory viruses and 27 (55%) tested RV positive. These 49 patients had similar PICU LOS and hospital LOS compared to patients without respiratory complaints on admission but had significantly longer non-invasive ventilatory support median 17.5 (4.5-38.5) versus 42 (18-73) hours, respectively, p=0.028) and post-extubation stridor significantly more often than patients who did not have respiratory complaints on admission (18 versus 5%, respectively, p=0.012).

As mild respiratory complaints on admission alone were not associated with worse clinical outcome (defined by duration of MV > 2 days or PICU LOS $\geq$ 4 days), we performed multivariate linear regression analysis to determine if a combination of a positive questionnaire, current complaints, abnormal laryngoscopy and RV positive would be predictive of prolonged PICU LOS. However, it was not possible to identify/develop a prediction model based on our results.

Twenty-six patients (15%) developed symptoms suspected for a postoperative respiratory infection. They had significantly longer cardiopulmonary bypass (CPB) times, cross-clamp times, higher PRISM and RACHS scores, more frequent steroid use during operation, and delayed sternal closure more often, indicating more complicated surgeries, as compared to patients without a postoperative infection (data not shown). After correction for all these factors and age, this group still had significantly increased PICU LOS (7 versus 2 days, p =<0.001), and hospital LOS (8 versus 16 days, p=0.002), and prolonged duration of mechanical ventilation (2.8 versus 0.4 days, p =0.003) and compared to patients without clinical infection.

Of all 163 patients, 45 (28%) were still admitted at the PICU at day four, which was similar in RV positive and RV negative patients (16 versus 29, respectively, p=0.119, figure 1). Twenty-two (49%) of these 45 patients were still intubated, and in 17 (77%) patients an follow-up nasopharyngeal swab was obtained for the detection of respiratory viruses. Seven of these 17 patients were RV positive prior to surgery, of which 5/7 (71%) were again RV positive in the follow-up sample, no significant difference in Cq value was found, of which two patients had an infection with another RV type compared to pre-operative (RV-B52 prior to surgery and RV-A41 on day 4, and RV-A71 and RV-A1 respectively). Ten of these 17 patients were RV negative prior to surgery, of which 1/10 (10%) became RV positive in the follow-up sample (RVA9).

The mean Cq value on the day of operation of the 74 rhinovirus positive patients was 27 (range 16.2 to 34.8). Rhinovirus positive patients with a high viral load (Cq<25) and rhinovirus negative patients had comparable hospital length of stay (median 8 vs 9 days respectively, p=0.070, corrected for age

and steroid use). No significant differences were found between rhinovirus Cq<25 and> 25 in hospital length of stay (p=0.812, corrected for age and steroid use). Mixed infections or RV species were not associated with a difference in PICU or hospital LOS.

In 67 (84%) out of 80 RV positive samples RV could be typed, 61 day 1 samples and 6 day 4 samples. Of the 64 unique samples, the majority of the patients had RV species A (56%, 36/64), followed by species C (27%,17/64) and species B (17%,11/64). An overview of the RV genotypes within each species, as detected per month in the study period, is shown in figure 3.

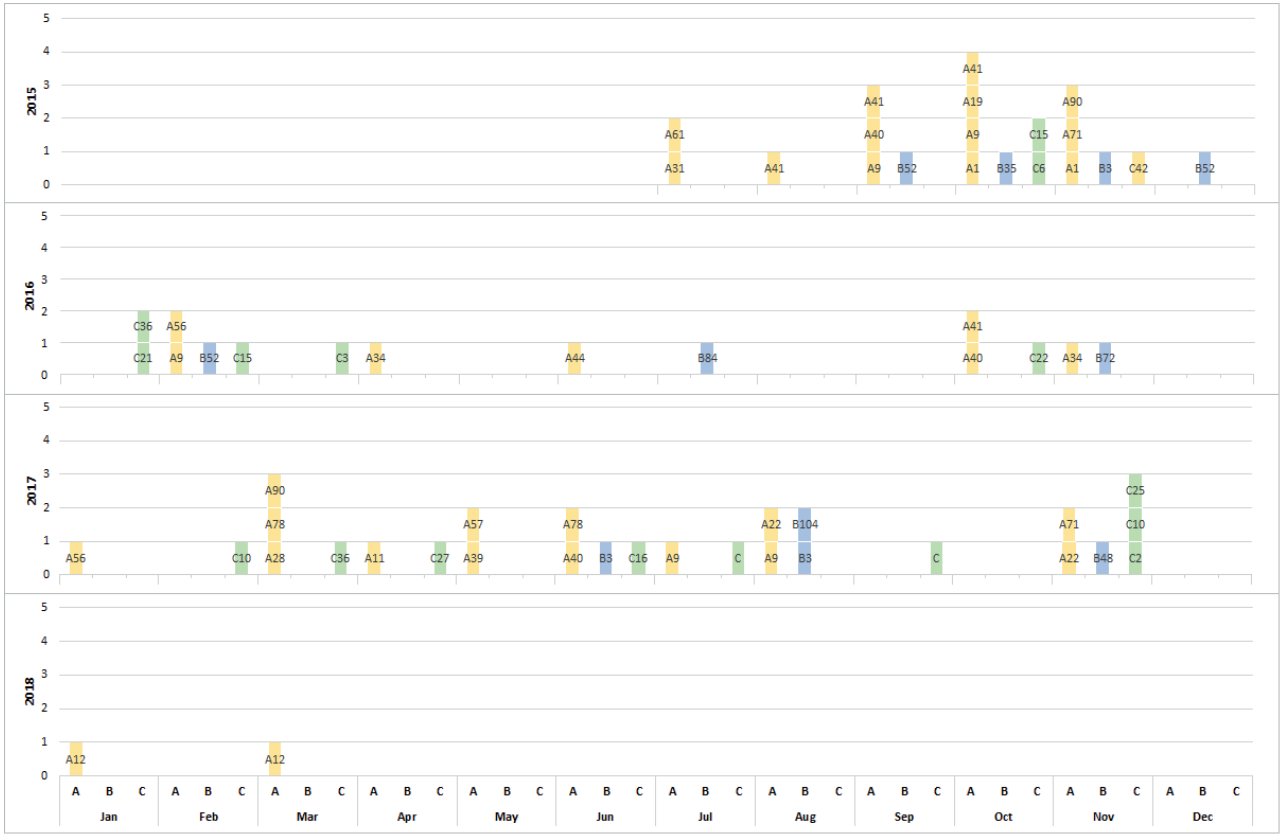

Figure 3. Rhinovirus genotypes per month.

Rhinovirus species per month during the study period are shown in different colors (species A yellow, species B blue and species C green). Genotypes within each species are shown, if available.

DISCUSSION

This prospective study, screening 163 children on the day of elective cardiac surgery during all seasons over several years, showed that RV was detected in 45% of the children and any respiratory virus even in 60%. Contrary to our hypothesis, RV positive patients had a similar duration of PICU LOS but a shorter hospital LOS compared to RV negative patients.

The very high proportion of 45% RV positive patients was unexpected. We predicted to find RV in approximately 20% of children, based on earlier reports of asymptomatic children in the Netherlands (range 14-28%) and infants undergoing cardiac surgery in Utah, USA[14,15]. We hypothesize that this high prevalence might be explained by our geographical location, inclusion throughout the year, young age (15 months), and underlying cardiac disease.

The earliest studies that demonstrated a negative effect of respiratory viruses on postoperative outcomes used ELISA's, in which positivity might have represented a more serious infection[5] compared to the modern highly sensitive PCR assays, which could represent prolonged shedding and asymptomatic carrier status[29-31]. However, we expected a certain number of asymptomatic carriers of RV to develop a symptomatic infection after surgery due to exposure to CPB and subsequent immunoparalysis[32-34]. Our results confirm a very recent and similar, but smaller, study by Delgado et al., who also observed no difference in postoperative outcomes in preoperatively tested (all respiratory viruses) asymptomatic infants[14].

We found a significantly shorter hospital length of stay in the RV positive patients compared to the RV negative patients and they were less often suspected of a postoperative clinical infection. This effect was also present in the 'any virus positive' patients. This might be the effect of an unknown confounder. However, recent studies investigating the relationship between respiratory microbiota and disease suggest that the microbiota acquired during childhood may affect immunological responses and may be related with health[35]. Rhinovirus can also very often be found in healthy children. In a study by Man et al. for instance, RV was significantly less common in children admitted with a lower respiratory infection than in healthy children[36].The precise mechanism as to how the respiratory viral and bacterial microbiota might be associated with health remains to be elucidated.

Almost all patients (96%) in our study had a positive questionnaire indicating respiratory symptoms in the 6 weeks prior to the operation. We deliberately asked parents about this 6-week period as the risk of peri-operative adverse events is increased up to 6 weeks after upper respiratory tract infections (URTI)[20]. Delgado-Corcoran et al. conducted a very similar questionnaire but only focused on two weeks pre-operatively and found a positive questionnaire in 66% of their patients, not related to clinical outcomes[14].

Thirty-one percent of the parents of patients in our study reported rhinorrhea and/or nasal congestion on admission. Parental confirmation of an URTI has been shown to be a better predictor of airway complications than the use of symptom criteria alone[37]. None had signs of active infection (fever, malaise, cough, etc.) and all were medically cleared for surgery. They did have significantly more post-extubation stridor requiring intervention (18 versus 5%; p=0.012). In a study by Malviya et al, children with preoperative signs of an URTI were also found to have more postoperative airway

complications[38]. Our results suggest that it is safe to operate children with rhinorrhea and/or nasal congestion, but the intensive care team should be aware of the higher chance of post-extubation stridor.

In our study, anesthesiologists reported redness and/or secretions on direct laryngoscopy in 31% of all patients, which was significantly associated with the development of a respiratory infection postoperatively but did not influence LOS. As far as we are aware, there is no literature about the relevance of laryngeal redness and/or secretions during elective intubation, although it might be possible that these could represent current mild URTI and might also lead to lower respiratory tract infections.

Patients who developed postoperative clinical signs of infection (16%) had significantly prolonged duration of mechanical ventilation, PICU LOS, and hospital LOS which is consistent with previous studies of children with symptomatic postoperative RV infections[2,4]. We could not identify pre-operative predictors of postoperative clinical infection. We seem to have a similar incidence of postoperative suspected infections compared to the study by Moynihan et al., who performed a PCR based on clinical suspicion of an infection in 18% (318/1737) of their patients following cardiac surgery in Queensland, Australia[4]. Twenty-three percent of their PCR's were virus positive compared to 45% in our cohort. Four percent of their entire cohort had a confirmed post-operative viral infection which is comparable to the 6% in our cohort.

Clinical RV infections tend to be more severe in patients with a higher viral load[39], however we did not find an association between viral load and our primary outcome measures though our study was not powered on comparison of subcategories.

The majority of the patients had a RV type A infections, which is the most prevalent species[40,41]. In this paper, although not powered to detect a difference, the different species were not associated with prolonged PICU LOS. Although previously RV-C was often linked to more serious disease in children, more recent publications do not confirm these findings[42-46]. Future work is needed to determine the optimal rhinovirus genotyping sequencing strategy in the light of recent studies using whole genome sequencing for viral typing[47].

Our study has limitations. First, we had a large number of exclusions, which might have introduced a selection bias. However, the intended sample size was reached and we included children during all months over several years minimizing potential bias. The limited number of patients operated in August and December might be explained by the holidays, in which elective operations are performed less. The second limitation is the lack of standardized pre-operative assessment. To reflect current standard of care we left the decision to clear patients for surgery at the discretion of the medical team. Unfortunately, we do not have data on the number of postponed surgeries. Third, defining an infection in children remains contentious and therefore we based our incidence of postoperative infection on the clinical judgment of the treating intensive care team rather than on set criteria, which does reflect the reality of PICU care. Fourth, we only collected PICU details of the first four days, which may have led to missing data. However, all relevant data regarding the primary

and secondary endpoints were available. Finally, being a single-center study, results might not be applicable to other centers.

We performed the largest, statistically powered, prospective observational study of pre-operative respiratory PCR testing in children undergoing cardiac surgery to date, with as main finding that RV positivity did not negatively impact PICU LOS.

Conclusions

Rhinovirus PCR positivity is highly common in asymptomatic children undergoing cardiac surgery in the Netherlands and is not associated with prolonged PICU LOS, but possibly even with shorter hospital LOS. Our findings do not support the use of routine testing for respiratory viruses in asymptomatic children admitted for elective cardiac surgery.

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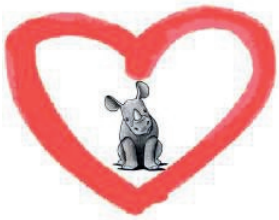
SUPPLEMENTARY FILES

File 1: Questionnaire, which was sent to all patients, asking for signs and symptoms of current and/or recent respiratory infections, see supplementary file study protocol

File 2: Case report form (CRF) for the anesthesiologist during the operation

File 3: Table Baseline characteristics of any virus positive/ negative patients

RISK STUDY



ANESTHESIOLOGY STUDY FORM

Operation date: ……

Weight: ……. (kg)

Induction of anesthesia:

o propofol
o sevoflurane
o esketamine
o other:……..

PATIENT STUDY STICKER

Anesthesia maintenance:

o propofol
o sevoflurane
o sufentanil
o remifentanil
o esketamine
o other: ……

Intubation:

o secretions? □ yes □ no
o redness / swelling? □ yes □ no
o pus? □ yes □ no
o endotracheal tube size: …… mm; cuff inflated: □ yes □ no
o details:……
o ventilation difficulties? □ no □ yes: ……..

nasopharyngeal swab obtained: □ yes □ no

blood products administered?:

o erythrocytes:……..ml
o thrombocytes:……ml
o plasma:……..ml

Bypass

- Dexamethasone? □ no □ yes, dose: ……..
- X-Clamping duration: ……..(min)
- CP-Bypass duration: ……(min)
- antegrade cerebral perfusion? □ no □ yes, duration: …… (min)

Other details:

……

S3 Table Baseline characteristics of any virus positive/negative patients

	Any virus positive n=97	Virus negative n=66	p-value
Demographics			
Age at surgery (months) (median, IQR)	19 (6-47)	10 (3-32)	0.510
Male (%)	45 (46)	36 (55)	0.307
Weight at surgery (kg) (median, IQR)	10.7 (7.1-16.1)	7.8 (5.5-12.1)	0.016
Underlying respiratory conditions (%)	14/94 (15)	8/66 (12)	0.598
Asthma	3 (21)	1 (13)	
Bronchitis	2 (14))	0	
Tracheomalacia	2 (14)	0	
Pleural fluid	0	1 (13)	
Multiple airway infections	3 (21)	0	
Other	4 (29)	6 (75)	
Infectious respiratory complaints			
Respiratory complaints past 6 weeks (%)	90/94 (96)	63 (95)	1.000
Current respiratory complaints (%)	35/94 (37)	14 (21)	0.030
Risk assessment			
PRISM score at admission (median, IQR) (II)	5 (3-9)	6 (3-10)	0.492
PRISM III (median, IQR)	3 (1-3)	3 (3-4)	0.107
PIM (mean, SD)	-3.77(0.60)	-3.56 (0.64)	0.073
Prematurity	11/94 (12)	8 (12)	0.936
Passive smoking	9/94 (10)	3 (5)	0.362
Operation			0.342
- Univentricular	10(10)	4(6)	
- Biventricular	87 (90)	62 (94)	
- CPB duration (minutes) (median, IQR)	87 (58-124.2)	97 (59-126.8)	0.722
- Cross clamp time (minutes) (median, IQR)	49 (25-91)	64.5 (36.2-94.8)	0.191
RACHS score (%)			0.037
1	24 (25)	6 (9)	
2	53 (55)	48 (73)	
3	14 (14)	10 (15)	
4	1 (1)	1 (2)	
Total blood products (median, IQR)	292 (200-375)	257 (195-330)	0.275
Cumulative Fluid (median, IQR)	957 (552.8-1290)	973 (604.5-1365)	0.531
Steroids during operation (%)	31 (32)	14 (21)	0.136
Number of inotropes (%)			0.756
0	3	2	
1	34	19	
2	28	24	
3	3	1	
4	1	0	
Delayed sternal closure (%)	7 (7)	4 (6)	1.000

IQR: interquartile range, PRISM: pediatric risk of mortality score, PIM: pediatric index of mortality, CPB: cardiopulmonary bypass, RACHS: risk adjustment for congenital heart surgery.