Airway ciliary dysfunction: Association with adverse postoperative outcomes in nonheterotaxy congenital heart disease patients

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ABSTRACT

Objective: Heterotaxy (HTX) congenital heart disease (CHD) patients with ciliary dysfunction (CD) have been shown to have increased postoperative respiratory morbidity. We hypothesized that non-HTX CHD infants with CD also will have increased postoperative morbidity, particularly respiratory complications.

Methods: Sixty-three infants with non-HTX CHD undergoing cardiac surgery were enrolled. Tests commonly used to assess for CD, nasal nitric oxide (nNO) measurements and nasal epithelial ciliary motion (CM) assessment, were obtained. Baseline characteristics and postoperative outcomes were collected and analyzed.

Results: Non-HTX CHD infants exhibited a high prevalence of abnormal CM (32%) and low nNO (39%). This finding was not correlated with demographics or surgical complexity. Infants with abnormal CM had increased odds of requiring noninvasive positive pressure ventilation (odds ratio [OR], 6.5; 95% confidence interval [CI], 1.5-29.4; $P = .016$) and respiratory medication use (OR, 4.4; 95% CI, 1.5-13.3; $P = .01$). In contrast, infants with low nNO showed evidence of abnormal pre- and postoperative systolic function (40% vs 4%; $P = .004$, and 34% vs 13%; $P = .056$, respectively) and had greater odds of acquiring infections (OR, 4.9; 95% CI, 1.4-17; $P = .014$).

Conclusions: Non-HTX CHD infants with abnormal CM showed increased postoperative morbidity associated with poor respiratory outcomes. In contrast, low nNO correlated with reduced hemodynamic function. These findings suggest screening for abnormal CM may allow perioperative interventions to reduce pulmonary morbidities. Whether low nNO may prognosticate poor hemodynamic function warrants further investigation. (J Thorac Cardiovasc Surg 2018;155:755-63)

Ciliary axoneme of a human nasal epithelial cell visualized by immunostaining.

Central Message

Airway ciliary dysfunction is associated with increased pulmonary morbidity after congenital cardiac surgery in infants with nonheterotaxy congenital heart disease.

Perspective

Current evidence suggests airway ciliary dysfunction in congenital heart disease (CHD) patients with heterotaxy is associated with increased postoperative pulmonary morbidity. Here, analysis of patients with a broad spectrum of nonheterotaxy CHD yielded similar findings. These results suggest that preoperative screening for ciliary dysfunction may allow therapeutic interventions to improve outcomes for at-risk CHD patients.

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The survival of patients with critical congenital heart disease (CHD) has improved greatly with surgical advances and improvements in critical care. However, postoperative morbidity remains a significant problem, with respiratory complications being a substantial burden. A mechanistic link between CHD and the disturbance of airway clearance

Scanning this QR code will take you to a supplemental videos, tables, and appendix for the article.

Abbreviations and Acronyms

CD	= ciliary dysfunction
CHD	= congenital heart disease
CICU	= cardiac intensive care unit
CM	= ciliary motion
HTX	= heterotaxy
LOS	= length of stay
NIPPV	= noninvasive positive pressure ventilation
nNO	= nasal nitric oxide
NO	= nitric oxide
PCD	= primary ciliary dyskinesia
PLOS	= postoperative length of stay

was suggested with the recent realization that motile cilia are required for both airway mucociliary clearance and embryonic development of visceral organ asymmetry, including the cardiovascular system.¹⁻⁴ Indeed, previous studies have shown CHD patients with heterotaxy (HTX), a birth defect involving randomized left-right patterning, have a high prevalence of motile ciliary dysfunction (CD), with those exhibiting CD suffering increased postoperative morbidity.⁵⁻⁷

CD in HTX CHD patients was identified by measuring nasal nitric oxide (nNO) and obtaining nasal epithelial scrapes for ciliary videomicroscopy assessment, 2 tests commonly used to evaluate for primary ciliary dyskinesia (PCD), a sinopulmonary disease associated with very low nNO and immotile or dyskinetic airway ciliary motion (CM).⁸ With these same tests, a study was conducted on outpatients with a broad spectrum of both HTX and non-HTX CHD, revealing a similarly high prevalence of CD (combination of both abnormal CM and low nNO in 33% of HTX CHD and 17% of non-HTX CHD).⁹ CD in these patients was associated with increased sinopulmonary symptoms, with ciliary function status shown to be more important than HTX status for determining the risk of manifesting sinopulmonary symptoms.⁹

In this current study, we investigated the hypothesis that non-HTX CHD patients with CD have increased postoperative morbidity and more respiratory complications. With previous evidence that CHD patients with CD could have abnormal CM with normal nNO or low nNO with normal CM,⁹ we further examined whether abnormal CM versus low nNO posed different risks for postoperative morbidity.

METHODS**Patient Recruitment**

Patients younger than 1 year of age with non-HTX CHD were prospectively enrolled from 2010 to 2014 at Children's Hospital of Pittsburgh of the University of Pittsburgh Medical Center (Pittsburgh, Pa). This age group was chosen to minimize heterogeneity in the study population. This infant cohort is a subset of a larger database containing patients of all ages with all types of CHD. Informed consent was obtained from parents

or guardians of patients. The study protocol was approved and performed in accordance with the Institutional Review Board of the University of Pittsburgh (PRO09090021; approved December 14, 2009, renewed September 1, 2016).

Inclusion Criteria

CHD patients recruited in this study comprised those with malformations of the heart and/or great arteries without laterality defects. All non-HTX CHD patients who had at least 1 cardiac surgery before 1 year of age were eligible for inclusion in the study. Patients hospitalized greater than 3 months before surgical repair or those with iatrogenic airway injury were excluded from the study. Minor surgical encounters including thrombectomy, isolated vessel reconstruction, sternal closure, and extracorporeal membrane oxygenation cannulation and decannulation were excluded from analysis as independent surgical events.

nNO Measurement

nNO measurements were obtained preoperatively with a CLD 88 sp (Eco Physics AG; Ann Arbor, Mich) nitric oxide (NO) analyzer according to established protocols with the tidal breath sampling technique.^{10,11} Infants were classified as having normal or low nNO based on the published prediction interval of normative infant nNO values.¹²

CM Analysis

Nasal epithelial tissue was obtained using rhino-probe curettage of the inferior nasal turbinate during the preoperative period. CM was examined using high-speed videomicroscopy via methods previously described.^{6,9} To rule out CM defects secondary to environmental insults like infection or allergy, nasal epithelial tissue was placed into culture after videomicroscopy for expansive growth and reciliation as described previously.⁹ The reciliated tissue was examined by videomicroscopy to assess whether CM was normal or abnormal (Appendix 1 and Videos 1 and 2). In cases in which the initial scrape and reciliation results were discordant, the CM status was determined from the reciliation data.

Outcome Data Collection

We collected data from each major surgical encounter a patient experienced over the study period. All clinical staff were blinded to the patients'

VIDEO 1. Nasal epithelial cells from a patient with normal ciliary motion. These nasal epithelial cells bear abundant cilia with long, synchronized, coordinated strokes. Note the ability of the beating cilia to move pericellular debris. Video available at: [http://www.jtcvsonline.org/article/S0022-5223\(17\)31968-2/fulltext](http://www.jtcvsonline.org/article/S0022-5223(17)31968-2/fulltext).

VIDEO 2. Nasal epithelial cells from a patient with abnormal ciliary motion. These nasal epithelial cells have stiff, dyskinetic, wavy cilia with uncoordinated strokes. Video available at: [http://www.jtcvsonline.org/article/S0022-5223\(17\)31968-2/fulltext](http://www.jtcvsonline.org/article/S0022-5223(17)31968-2/fulltext).

CM and nNO assessments. Detailed demographic, surgical, and postoperative outcome data were abstracted from the electronic medical record using patient discharge summaries, progress notes, operative reports, and daily flowsheets. Basic demographic data were collected, including age at nNO and CM sampling, sex, race, and gestational age. Medical history and preoperative data were gathered including an anatomic description of CHD, genetic evaluation (Appendix 1), comorbidities, age at surgery, preoperative weight, and preoperative systemic systolic ventricular function. Surgical mortality and risk adjustment scores were collected, including the Society of Thoracic Surgeons and European Association for Cardio-Thoracic Surgery Congenital Heart Surgery Database categories, Aristotle Basic Complexity score (Aristotle), and Risk Adjustment in Congenital Heart Surgery score.¹³⁻¹⁵

Length of stay (LOS) parameters collected included postoperative length of stay (PLOS), initial cardiac intensive care unit (CICU) LOS, and total CICU LOS, which included additional time spent in CICU due to readmission after initial transfer to the step-down unit. Ventilation parameters collected included mechanical ventilation days, noninvasive positive pressure ventilation (NIPPV) days, and supportive ventilation days, which included high-flow nasal cannula and nasal cannula. Duration of postoperative medication requirements were recorded after each major surgical encounter.

Data regarding postoperative complications were gathered, including reintubations, development of pleural effusions and/or pneumothoraces, necrotizing enterocolitis, and infections. Failed extubation was defined as a patient being reintubated less than 24 hours after extubation. Pleural effusions were only counted if they required chest tube drainage. Intrinsic airway disease was noted for patients with endoscopic evidence of external or internal airway compression or vocal cord dysfunction. Pneumothorax was defined as clinical symptoms with radiographic evidence of air in the pleural space. Diaphragm paresis was noted for patients with sonographic or fluoroscopic evidence of paresis. Qualitative systemic ventricular function was abstracted from transthoracic echocardiography assessments by pediatric cardiologists who were blinded to all aspects of the study. Viral infection was defined as presence of clinical symptoms and positive nasal viral panel. Bacterial infection was defined as presence of clinical symptoms and positive blood culture and excluded pneumonia and tracheitis. Fungal infection was defined as presence of clinical symptoms and positive fungal culture. Pneumonia (bacterial) was defined as clinical symptoms, the presence of infiltrate on chest radiograph, and infectious disease recommendation to treat with antimicrobials for bacterial

pneumonia. Tracheitis was defined as clinical symptoms and positive tracheal culture.

Statistical Analysis

Summary statistics such as proportion and median with interquartile range were used to describe patient characteristics and their postoperative outcomes. For patient characteristics, the χ^2 test was used to compare categorical variables and the Fisher exact test was applied when appropriate, and Wilcoxon rank-sum test was used to compare continuous variables between subgroups. For baseline surgical characteristics and postoperative outcomes, mixed-effects models were applied to adjust for the intrasubject correlation among multiple surgical events of individual patients. As the postoperative continuous outcomes were right-skewed, the linear mixed models for repeated measures were used to analyze the effect of CM and nNO on the log-transformed continuous outcomes that were approximately normal after transformation. The generalized linear mixed models for repeated measures were used to assess the differences in the categorical outcomes between the abnormal and normal CM or nNO subgroups. Multivariate analysis of adverse postoperative outcomes based on generalized linear mixed models was performed to examine the effect of CM or nNO, controlling for baseline surgical characteristics (age, weight, surgical severity, ventricular function, and single-ventricle status). All tests were 2-sided, and a P value $\leq .01$ was considered statistically significant. A more conservative significance level of .01 was used to adjust for multiple comparisons. Statistical analyses were performed with SAS 9.4 (SAS Institute Inc, Cary, NC) and STATA 12.1 (STATA Corp, College Station, Tex).

RESULTS

Baseline Demographics

We recruited 63 infants with a broad spectrum of critical non-HTX CHD (Figure 1 and Table E1). Two patients had 1 of 2 surgical encounters excluded: One for hospitalization >3 months before surgical repair and the other for iatrogenic tracheal injury. During the study period, the survival rate was 99%, with only 1 patient death reported in the immediate postoperative period, precluding analysis of postoperative mortality.

FIGURE 1. Spectrum of CHD in study participants. The distribution of non-HTX CHD observed in study participants is summarized in this pie chart. “Other” category includes 1 patient each with pulmonary atresia/intact ventricular septum, isolated infradiaphragmatic total anomalous pulmonary venous return, double-outlet left ventricle, critical aortic stenosis, complete atrioventricular septal defect, and anomalous left coronary artery from the pulmonary artery. *d-TGA*, d-Transposition of the great arteries; *HLHS*, hypoplastic left heart syndrome; *ToF*, tetralogy of Fallot; *DORV*, double-outlet right ventricle; *DILV*, double-inlet left ventricle; *ASD/VSD*, atrial septal defect/ventricular septal defect.

FIGURE 2. Summary of ciliary motion and nasal nitric oxide findings in non-HTX CHD patients. A, Distribution of normal/abnormal CM and nNO in all non-HTX CHD patients. B, Distribution of normal/abnormal CM and nNO in non-HTX CHD patients with both CM and nNO data. CHD, Congenital heart disease; CM, ciliary motion; nNO, nasal nitric oxide; Abn, abnormal; Nml, normal.

The most common cardiac abnormalities in study participants were d-transposition of the great arteries (14 patients) and hypoplastic left heart syndrome (11 patients). Thirty-six (57%) patients exhibited conotruncal defects (Table E2). The majority of the patients were male (67%), born at full term (97%), and white (87%), with the remainder being African American (6%), Hispanic (3%), Arab (2%),

and Asian (2%) (Table E2). Genetic evaluation, carried out by microarray analysis as the standard of care (Appendix 1), was abnormal in 24% of study participants (Table E2). Detailed information regarding anatomic subtypes of CHD, comorbidities of each patient, and genetic abnormalities is provided in Table E1.

Baseline Demographics Stratified by CM and nNO Status

Nasal scrapes were obtained in 59 (94%) patients for CM analysis, and nNO measurements were performed in 56 (89%) (Figure 2, A, and Table E3). Abnormal CM was observed in 32% of the patients. Low nNO was observed in 39% of the patients. For patients (N = 52) with both CM and nNO assessments, 6% had both abnormal CM and low nNO (Figure 2, B, and Table E3). There was no significant difference in recruitment age or prematurity, sex, race, or presence of an abnormal genetic evaluation in patients with abnormal versus normal CM and low versus normal nNO (Table E2). Patients with low nNO were recruited at an older age than patients with normal nNO (24 [6-53] days vs 5 [3-21] days, respectively; $P = .038$). Conotruncal defects were significantly more common in patients with abnormal CM (84% vs 48%, $P = .01$).

TABLE 1. Baseline surgical parameters in CHD patients by ciliary motion and nasal nitric oxide

	All surgical events (n = 95)	Events with CM data (n = 91)		Events with nNO data (n = 83)	
		Normal (n = 58)	Abnormal (n = 33)	Normal (n = 48)	Low (n = 35)
Age at surgery, d	14 (7-91)	11 (6-84)	30 (9-127)	9 (7-92)	30 (8-91)
		$P = .060$		$P = .23$	
Preoperative weight, kg	3.7 (3.1-5.0)	3.4 (3.1-4.7)	4.3 (3.2-6.1)	3.7 (3.1-4.9)	3.5 (3.1-5.1)
		$P = .054$		$P = .99$	
STS-EACTS category*	3 (2-4)	3 (2-4)	3 (2-4)	3 (2-4)	3 (2-4)
		$P = .65$		$P = .47$	
Abn PreOp Sys Fxn, n (%)	21 (22)	13 (22)	7 (21)	2 (4%)	14 (40)
		$P = .64$		$P = .004$	
Single-ventricle palliation, n (%)	40 (42)	30 (52)	10 (30)	14 (29)	20 (57)
		$P = .15$		$P = .061$	
Surgeon 1, n (%)	68 (72)	41 (71)	24 (73)	34 (71)	26 (74)
		$P = .87$		$P = .51$	
CPB time, min	88 (62-115)	89 (66-112)	89 (55-134)	86 (57-120)	91 (65-108)
		$P = .32$		$P = .96$	
XC time, min	27 (0-53)	23 (0-44)	44 (0-54)	30 (0.8-55)	26 (0-46)
		$P = .36$		$P = .54$	
DHCA, n (%)	28 (30)	21 (36)	7 (21)	14 (29)	11 (31)
		$P = .15$		$P = .83$	

Data are median (interquartile range) or n (%). P values obtained from the linear mixed models and generalized linear mixed model for continuous and categorical variables, respectively. CM, Ciliary motion; nNO, nasal nitric oxide; STS-EACTS, Society of Thoracic Surgeons-European Association for Cardio-Thoracic Surgery; Abn PreOp Sys Fxn, abnormal preoperative systolic ventricular function of systemic ventricle; CPB, cardiopulmonary bypass; XC, aortic crossclamp; DHCA, deep hypothermic circulatory arrest. *Empirically based mortality stratification, categories of 1-5 with greater numbers implying greater mortality risk, based on of the STS-EACTS Congenital Heart Surgery Database.

TABLE 2. Association between abnormal CM and postoperative outcomes

	All surgical events (n = 95)	Surgical events for patients with CM data (n = 91)		
		Normal CM (n = 58)	Abnormal CM (n = 33)	P value
Postoperative LOS, d	15 (10-24)	16 (11-26)	13 (6-19)	.057
Total CICU LOS, d	8 (5-11)	8 (5-11)	7 (4-10)	.19
Initial CICU LOS, d	7 (5-11)	8 (5-11)	7 (4-10)	.19
Abn PostOp Sys Fxn, n (%)	22 (23)	15 (26)	6 (18)	.37
ECMO, n (%)	10 (11)	6 (10)	4 (12)	.8
Delayed sternal closure, n (%)	58 (61)	39 (67)	17 (52)	.15
Mechanical ventilation, n (%)	84 (88)	50 (86)	30 (91)	.58
Supportive ventilation, n (%)	88 (93)	53 (91)	31 (94)	.66
NIPPV, n (%)	24 (25)	9 (16)	14 (42)	.012
Reintubation, n (%)	15 (16)	7 (12)	8 (24)	.27
Respiratory medication (any) use, n (%)	31 (33)	11 (19)	17 (52)	.004
Albuterol use, n (%)	22 (23)	8 (14)	14 (42)	.007
Dornase alfa use, n (%)	3 (3)	0 (0)	2 (11)	.10*
Ipratropium use, n (%)	3 (3)	0 (0)	3 (16)	.03*
Overall infection, n (%)	28 (30)	15 (26)	12 (36)	.3
Bacterial infection, n (%)	25 (26)	14 (24)	11 (33)	.35
Viral infection, n (%)	4 (4)	0 (0)	3 (16)	.03*

Data are median (interquartile range) or n (%). P values obtained by from the linear mixed models and generalized linear mixed model for continuous and categorical variables, respectively. CM, Ciliary motion; LOS, length of stay; CICU, cardiac intensive care unit; Abn PostOp Sys Fxn, abnormal postoperative systolic ventricular function of systemic ventricle; ECMO, extracorporeal membrane oxygenation; NIPPV, noninvasive positive pressure ventilation. *P value by the Fisher exact test for testing whether the percentages are difference between the normal CM (n = 40) and abnormal CM (n = 19) patients.

Preoperative Surgical Data

The 63 CHD infants recruited into this study collectively had 95 surgical encounters available for analysis. The median age of recruitment was 10 [3-29] days (Table E2). The median age at surgery was 14 [7-91] days (Table 1). Single-ventricle palliation was performed in 40 (42%) patient encounters, with surgeon 1 (of 2) performing 68 (72%) of the surgical cases.

Of the 95 surgical events, 36% occurred in patients with abnormal CM, 42% in patients with low nNO, and 8% in patients with both abnormal CM and nNO. There was no difference in preoperative weight, Society of Thoracic Surgeons–European Association for Cardio-Thoracic Surgery category, Risk Adjustment in Congenital Heart Surgery or Aristotle scores, surgeon, median cardiopulmonary bypass time, crossclamp time, or deep hypothermic circulatory arrest time among patients stratified either by CM or nNO status (Table 1). There was a trend toward younger age at surgery in patients with normal CM (11 [6-84] days vs 30 [9-127] days; $P = .06$). No difference was found in preoperative systolic function by CM status. However, abnormal systolic function was significantly associated with low nNO (40% vs 4%; $P = .004$) (Table 1).

Postoperative Outcome Assessments

Our patient cohort had a median PLOS of 15 [10-24] days (Table 2). The median total CICU LOS was 8 [5-11] days,

and initial CICU LOS was 7 [5-11] days. In patients with low nNO, initial CICU LOS (10 [5-12] days vs 7 [4-8] days; $P = .042$) and total CICU LOS (10 [5-12] days vs 7 [4-9] days; $P = .041$) were longer (Table 3). These low nNO patients also had a greater prevalence of decreased postoperative systolic function (34% vs 13%; $P = .056$). The decreased systolic function in the low nNO patients persisted in 9 of 16 patients up to the time of hospital discharge. Low nNO also was correlated with an increase in number of days treated with milrinone (6 [4-8] days vs 4 [3-6] days; $P = .021$), dexmedetomidine (6 [3-8] days vs 3 [2-6] days; $P = .002$), and nicardipine requirement (34% vs 6%; $P = .034$) (Table 3). All other postoperative medications used, including inhaled NO, were not different among patients with normal or low nNO (Table E4).

Patients with abnormal CM showed no difference in total or initial CICU LOS or postoperative systolic function. Neither abnormal CM nor low nNO was correlated with differences in PLOS, days of extracorporeal membrane oxygenation, or CICU readmission (Tables 2 and 3). We also found no correlation between the incidence of necrotizing enterocolitis or use of a nasogastric tube for nutrition at hospital discharge stratified by CM or nNO status (Table E5). Patients exhibiting both abnormal CM and low nNO comprised only 3 patients and 6 surgical events, precluding any meaningful analysis of outcome parameters in this small subset of patients.

TABLE 3. Association between low nNO and postoperative outcomes

	All surgical events (n = 95)	Surgical events for patients with nNO data (n = 83)		
		Normal nNO (n = 48)	Low nNO (n = 35)	P value
Postoperative LOS, d	15 (10-24)	15 (10-20)	18 (10-29)	.28
Total CICU LOS, d	8 (5-11)	7 (4-9)	10 (5-12)	.041
Initial CICU LOS, d	7 (5-11)	7 (4-8)	10 (5-12)	.042
Abn PostOp Sys Fxn, n (%)	22 (23)	6 (13)	12 (34)	.056
ECMO, n (%)	10 (11)	6 (13)	3 (9)	.58
Delayed sternal closure, n (%)	58 (61)	28 (58)	23 (66)	.50
Mechanical ventilation, n (%)	84 (88)	42 (88)	31 (89)	.91
Supportive ventilation, n (%)	88 (93)	46 (96)	32 (91)	.42
NIPPV, n (%)	24 (25)	11 (23)	8 (23)	.92
Reintubation, n (%)	15 (16)	6 (13)	7 (20)	.39
Respiratory medication (any) use, n (%)	31 (33)	13 (27)	12 (34)	.50
Dexmedetomidine, d	4 (2-7)	3 (2-6)	6 (3-8)	.002
Fentanyl, d	3 (2-5)	3 (2-5)	4 (3-6)	.098
Milrinone, d	5 (3-7)	4 (3-6)	6 (4-8)	.021
Nicardipine use, n (%)	17 (18)	3 (6)	12 (34)	.034
Overall infection, n (%)	28 (30)	8 (17)	16 (46)	.01
Bacterial infection, n (%)	25 (26)	8 (17)	13 (37)	.048
Viral infection, n (%)	4 (4)	1 (2)	1 (3)	.83

Data are median (interquartile range) or n (%). P values were obtained from the linear mixed models and generalized linear mixed model for continuous and categorical variables, respectively. *nNO*, Nasal nitric oxide; *LOS*, length of stay; *CICU*, cardiac intensive care unit; *Abn PostOp Sys Fxn*, abnormal postoperative systolic ventricular function of systemic ventricle; *ECMO*, extracorporeal membrane oxygenation; *NIPPV*, noninvasive positive pressure ventilation.

Postoperative Respiratory Outcome Assessments

There was no difference observed for the requirement of mechanical ventilation or other supportive ventilation, including high-flow nasal cannula and nasal cannula among patients with abnormal CM, but the requirement of NIPPV was greater (42% vs 16%; $P = .012$) (Table 2). A significantly greater use of respiratory medications, inclusive of inhaled β -agonists, inhaled corticosteroids, mucolytics, and lung surfactant, was observed in patients with abnormal CM (52% vs 19%; $P = .004$) (Table 2). Multivariate analysis also indicated that the odds of requiring NIPPV, receiving β -agonist therapy, and receiving any type of respiratory medication were significantly greater in patients with abnormal CM compared with those for infants with normal CM (Table 4). In contrast, none of these parameters were significantly changed in patients with low nNO. Mechanical ventilation days, NIPPV, duration of supportive ventilation, and respiratory medication use were not affected by low nNO (Table 3). Neither abnormal CM nor low nNO correlated with changes in the rate of reintubation, failed extubation, pneumothorax, incidence of diaphragm paralysis or tracheostomy, presence of intrinsic airway disease, presence of effusion, number of chest tube days, or the amount of chest tube drainage (Tables 2 and 3; Table E5).

Postoperative Infection Outcomes

A greater proportion of patients with abnormal CM had respiratory viral infections (3 of 19 patients [16%] vs 0%; $P = .03$) (Table 2), but no difference was observed for bacterial infection or combined infection incidence (bacterial, viral, fungal infections, pneumonia, tracheitis) (Table 2 and Table E5). In contrast, patients with low nNO had an increased incidence of bacterial infections (37% vs

TABLE 4. Multivariate analysis of CM and nNO for adverse postoperative outcomes

Outcomes	Adjusted odds ratio (95% CI)	P value
Abnormal vs normal CM		
NIPPV	6.53 (1.47-29.4)	.016
β -Agonist use	5.78 (1.60-20.8)	.009
Respiratory medication use	4.42 (1.47-13.3)	.01
Low vs normal nNO		
Overall infection	4.90 (1.42-17.0)	.014
Bacterial infection	2.95 (0.82-10.6)	.094

For each postoperative outcome, a generalized linear mixed model with the logit-link was carried out to estimate the adjusted odds ratio for the CM or nNO group, controlling for the baseline characteristics (age at surgery, preoperative weight, STS-EACTS category, abnormal preoperative systolic ventricular function, and surgical classification). *CI*, Confidence interval; *CM*, ciliary motion; *NIPPV*, noninvasive positive pressure ventilation; *nNO*, nasal nitric oxide.

17%; $P = .048$) (Table 3) with an increased combined infection incidence compared with those with normal nNO (46% vs 17%; $P = .01$) (Table 3). Because viral infections were seen only in patients with abnormal CM, this precluded the ability to calculate an odds ratio. Those with low nNO had nearly 5 times the odds of acquiring an infection (95% confidence interval, 1.42-17; $P = .014$) in the multivariate analysis, controlling for baseline characteristics (Table 4). The overall rate of fungal infections, bacterial pneumonia, and tracheitis was not different with abnormal CM or low nNO (Tables E5 and E6).

DISCUSSION

We found a high prevalence of abnormal airway CM and low nNO in non-HTX CHD infants undergoing cardiac surgery. Postoperative morbidity was significantly increased with abnormal CM or low nNO, with the postoperative parameters affected by abnormal CM differing from those affected by low nNO. Despite CM assessment and nNO measurement being standard, validated tests for PCD, the mechanism linking the 2 are unknown, and there are reports of normal nNO levels in confirmed PCD patients.¹⁶⁻¹⁸ Hence, the divergence in our CM and nNO findings in CHD patients is not entirely surprising.

Abnormal CM Correlated With Increased Respiratory Morbidity in Non-HTX CHD Infants

We showed non-HTX CHD infants with abnormal CM exhibited a significant increase in respiratory morbidity, including increased length of NIPPV, greater need for respiratory medications, and increased incidence of viral respiratory infections. These findings were observed despite abnormal CM infants being older at the time of surgery and more likely to have a biventricular repair, both of which have been associated with decreased postoperative morbidity/mortality.^{19,20}

Despite not sharing classic features of PCD, CHD infants with abnormal respiratory CM appear to have mucociliary clearance defects that may account for their increased postoperative respiratory morbidity. This is suggested by increased use of respiratory medications, including β -agonists and inhaled corticosteroids, and the need for NIPPV. We plan to corroborate this finding in the future with direct assessments of mucociliary clearance in CHD infants with abnormal CM.

Our present study supports the previous work of Harden and colleagues,⁷ which showed increased postoperative use of inhaled β -agonists in HTX infants with CD. The replication of this finding in our cohort of exclusively non-HTX CHD infants at an independent institution suggests abnormal CM has broader relevance for postsurgical outcomes in infants with critical CHD, irrespective of the presence of concomitant HTX.

Association of Abnormal CM Versus Low nNO With Infections

The increased incidence of bacterial infections in infants with low nNO is consistent with the known inhibitory effect of NO on microbial growth and its modulation of the immune/inflammatory response.^{21,22} NO is also known to stimulate ciliary beat frequency, suggesting low nNO might compromise mucociliary clearance function in the airway. In contrast, we found infants with abnormal CM had increased respiratory viral infections but not bacterial infections. This may reflect a propensity for viral entry via the ciliated epithelia, observations suggesting the cilium may be the point of viral entry.²³⁻²⁶ Yet another study showed FUZ, a protein required for ciliogenesis, is also required for endocytosis-mediated viral entry.²⁵

Low nNO Associated With Hemodynamic Alterations

Infants with low nNO exhibited a greater incidence of decreased systolic function immediately before surgery and at the time of hospital discharge. The low nNO preceded the decrease in systolic function, as the majority of infants exhibited normal systolic function at the time of nNO assessment, but their systolic function worsened by the time of surgery (median 8 days later). Abnormal systolic function in most of these infants persisted to the time of hospital discharge. Such infants also showed an increased need for intravenous supportive therapy and longer stay in the CICU. Interestingly, there was also a significant increase in use of systemic vasodilators in these infants. This finding raises the interesting possibility that defective vasoregulation may arise secondary to impaired endogenous NO production in such infants.

The alterations in hemodynamics seen in infants with low nNO are consistent with those reported by Yau and colleagues,²⁷ showing low nNO levels correlated with failing single-ventricle physiology and a trend toward adverse outcomes in CHD infants with single-ventricle lesions. The benefits of adequate NO bioavailability have been further illustrated by James and colleagues,²⁸ who showed a reduced incidence of postcardiopulmonary bypass low cardiac output syndrome in children who received supplemental NO via the bypass oxygenator during surgery. The mechanism driving the hemodynamic effects associated with low nNO is presently unknown. As cilia are thought to play a role in NO production and nonmotile primary cilia have been shown to mediate calcium signaling and mechanosensory signal transduction, CHD infants with normal motile cilia function may nevertheless have primary cilia defects that may contribute to abnormal hemodynamic regulation.^{29,30} Further studies are needed to investigate whether low nNO may be correlated with low systemic plasma NO species. Such studies will be needed to

elucidate the precise link between low nNO and reduction in systolic function.

Limitations

A limitation in our study is the relatively small sample size of infants, which may have underestimated or failed to detect differences in certain outcome parameters. Our results are based on an observation study of 63 non-HTX CHD infants; thus, the findings from comparisons of multiple outcomes are hypothesis generating and require validation in a larger cohort. It was particularly underpowered to detect a difference in outcome for infants with both abnormal CM and low nNO, or a mortality difference, given the very low mortality rate in our study population. Reciliation data were not available for all of the patients included in our study, and thus abnormal CM may have been somewhat overestimated. This may have biased our results toward the null hypothesis for outcome parameters that are more modestly affected by abnormal CM.

CONCLUSIONS

Non-HTX CHD infants with abnormal CM showed increased postoperative morbidity, with abnormalities in CM predictive of poor respiratory function. Those with low nNO were observed to exhibit hemodynamic perturbation. Longitudinal assessment is needed to examine more long-term outcomes of surgical palliation beyond the hospital admission period for the index surgical event. Further studies also are needed to investigate the mechanisms by which abnormal CM and low nNO drive the different outcome parameters. Together, these findings suggest preoperative evaluation for CD in CHD infants may identify at-risk infants, thereby providing opportunities for perioperative interventions to reduce postoperative morbidity. This could improve outcomes for infants with critical CHD, many of whom must undergo multiple high-risk cardiac surgeries for palliation of their structural heart disease.

Conflict of Interest Statement

Authors have nothing to disclose with regard to commercial support.

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Key Words: nitric oxide, congenital heart disease, postsurgical outcome, airway epithelia, respiratory cilia, primary ciliary dyskinesia

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APPENDIX 1. SUPPLEMENTAL METHODS AND RESULTS

Nasal epithelial tissue samples were obtained using Rhino-Probe curettage of the inferior nasal turbinate. High-speed videomicroscopy was performed and the tissue placed in culture. Upon culture, nasal epithelial cells lose their cilia, dedifferentiate, and undergo mitoses, whereupon they were grown to confluence. After confluence was achieved, cells were placed in suspension culture, allowed to reciliate, and videomicroscopy performed on the reciliated tissue. Ciliary motion (CM) of both the initial nasal scrape as well as reciliated tissue was evaluated by a panel of 3 investigators (C.W.L., M.Z., and O.K.) who were blinded to the patient phenotype and nasal nitric oxide values. Five to fifteen videos were reviewed for each sample/subject and CM classified as 1, normal; 2, probably normal; 3, probably abnormal; or 4, abnormal by consensus evaluation. To create a binary variable, those with scores of 1 or 2 were categorized as normal CM, and those with 3 or 4 were categorized as abnormal CM. Samples were not obtained on patients with evidence of ongoing respiratory disease. Whenever reciliation was possible, final determination of

CM was made based on findings from reciliated epithelial tissue, to exclude secondary (environmental, medication) causes of abnormal CM. Twenty-seven (43%) of 63 patients had successful reciliation of nasal epithelial cells. Three (11%) patients had a change in the classification of their ciliary motion after reciliation was performed.

Most patients underwent genetic evaluation with a comparative genomic hybridization + single-nucleotide polymorphism microarray^{E1} (constructed by the International Standards for Cytogenomic Arrays), which was performed at the Pittsburgh Cytogenetics Laboratory at Magee-Women's Hospital of University of Pittsburgh Medical Center. In patients with suspected genetic syndromes in whom a microarray was normal, a genetics consultation was obtained to determine the need for additional genetic testing. In 8% (5/63) patients, no genetic testing was available for review.

E-Reference

E1. Yatsenko SA, Davis S, Hendrix NW, Surti U, Emery S, Canavan T, et al. Application of chromosomal microarray in the evaluation of abnormal prenatal findings. *Clin Genet*. 2013;84:47-54.

TABLE E1. Clinical characteristics of recruited non-HTX CHD patients

ID #	Anatomic description	Comorbidities*	Genetics
7004	DILV, d-TGA, type A interrupted aortic arch	Bronchomalacia, seizures	Normal microarray
7027	d-TGA	Situs inversus totalis, seizures	Normal microarray
7035	ASD, VSD	Tethered cord, hypothyroidism	Multiple congenital anomalies with normal microarray
7040	ASD, VSD	Bilateral grade I vesicoureteral reflux	Normal microarray
7042	HLHS		Normal microarray
7053	d-TGA		Normal microarray
7057	d-TGA		Normal microarray
7058	ToF	Tethered cord, exotropia, neurogenic bladder, hydroureter	8p deletion, 13q12.11 duplication
7059	Variant AVSD		Normal microarray
7102	ToF-PA, MAPCAs	Hypocalcemia	Normal microarray
7113	DORV, partial AVSD		Normal microarray
7129	d-TGA		Normal microarray
7130	DOLV, malaligned VSD, aortic arch hypoplasia		Normal microarray
7134	DORV, subaortic VSD	Aspiration, laryngeal cleft	22q11 deletion (DiGeorge)
7146	HLHS	Developmental dysplasia of hips	1q.21 duplication
7151	d-TGA	Atopic dermatitis	Normal microarray
7168	DILV, l-TGA, coarctation		3p24.3 deletion
7190	ToF		Normal microarray
7199	HLHS	Hypothyroidism	20p11.21 duplication
7208	d-TGA	Duplicated renal collecting system	Normal microarray
7225	HLHS	Hypothyroidism	Normal microarray
7242	Truncus arteriosus		18p11.32 duplication
7253	Coarctation, VSD		No testing available
7266	Truncus arteriosus		No testing available
7281	d-TGA		10q21.3q22.1 deletion
7288	Truncus arteriosus	Hydronephrosis	Normal microarray
7295	d-TGA		No testing available
7302	HLHS		Normal microarray
7306	d-TGA	Ectopic kidney	Normal microarray
7319	DORV, subaortic VSD	Ectopic kidney	6p25.1p24.3 duplication
7336	d-TGA		Normal microarray
7351	HLHS	Bilateral renal dysplasia	16p13.3 deletion (Kabuki)
7374	TA, l-TGA	Supraventricular tachycardia	Normal microarray
7376	Truncus arteriosus	Micrognathia, subglottic stenosis	22q11 deletion (DiGeorge)
7379	ToF-absent pulmonary valve		Normal microarray
7380	Critical aortic stenosis		Normal microarray
7381	d-TGA		Normal microarray
7386	HLHS		Normal microarray
7389	HLHS		Normal microarray
7397	ToF-PA		Normal microarray
7400	HLHS		Normal microarray

(Continued)

TABLE E1. Continued

ID #	Anatomic description	Comorbidities*	Genetics
7401	DORV, d-TGA		Normal microarray
7405	Infradiaphragmatic TAPVR		Normal microarray
7409	TA, d-TGA, aortic arch hypoplasia	Laryngomalacia	No testing available
7411	Aortic arch hypoplasia	Intrauterine growth restriction	Normal microarray
7414	Aortic arch hypoplasia, partial AVSD		Normal microarray
7415	ToF-PA		Normal microarray
7421	ToF		Normal microarray
7422	d-TGA		Normal microarray
7425	Shone complex		Normal microarray
7428	Shone complex	Supraventricular tachycardia	Normal microarray
7433	d-TGA	Prematurity, chronic lung disease, grade II interventricular hemorrhage	Normal microarray
7437	PA-IVS		Normal microarray
7438	HLHS	Aspiration, hypospadias	Normal microarray
7441	DORV, subaortic VSD		Normal microarray
7444	DILV, l-TGA	Neuroirritability	2p16.3 microdeletion (NRNX1)
7447	DILV, straddling tricuspid valve		Normal microarray
7448	ALCAPA		No testing available
7449	DORV, PA, superior-inferior ventricles	Medical necrotizing enterocolitis	Normal microarray
7450	TOF-PA		
7458	d-TGA		Normal microarray
7459	Shone complex		Xp22.33q28 triplication (Triple X)
7466	HLHS	Medical necrotizing enterocolitis	Normal microarray

DILV, Double-inlet left ventricle; *d-TGA*, d-transposition of the great arteries; *ASD*, atrial septal defect; *VSD*, ventricular septal defect; *HLHS*, hypoplastic left heart syndrome; *ToF*, tetralogy of Fallot; *AVSD*, atrioventricular septal defect; *ToF-PA*, tetralogy of Fallot with pulmonary atresia; *MAPCAs*, multiple aortopulmonary collateral arteries; *DORV*, double-outlet right ventricle; *DOLV*, double-outlet left ventricle; *l-TGA*, l-transposition of the great arteries; *TA*, tricuspid atresia; *TAPVR*, total anomalous pulmonary venous return; *PA-IVS*, pulmonary atresia-intact ventricular septum; *ALCAPA*, anomalous left coronary artery off the pulmonary artery; *PA*, pulmonary atresia. *Comorbidities present at the time of patient recruitment.

TABLE E2. Demographic data for total patients and subcategorized by ciliary motion and nNO

	All patients (n = 63)	Ciliary motion (n = 59 patients)		nNO (n = 56 patients)	
		Normal (n = 40)	Abnormal (n = 19)	Normal (n = 34)	Low (n = 22)
Age at recruitment, d	10 (3-29)	9 (3-26)	7 (4-55)	5 (3-21)	24 (6-53)
		<i>P</i> = .42		<i>P</i> = .038	
Male sex, n (%)	42 (67)	27 (68)	15 (79)	26 (76)	13 (59)
		<i>P</i> = .55		<i>P</i> = .28	
White race, n (%)	55 (87)	36 (90)	15 (79)	30 (88)	20 (91)
		<i>P</i> = .42*		<i>P</i> > .99*	
Gestation >37 wk, n (%)	61 (97)	39 (98)	19 (100)	34 (100)	20 (91)
		<i>P</i> > .99*		<i>P</i> = .15*	
Normal genetic evaluation, n (%)	45 (76)	32 (80)	11 (58)	25 (74)	15 (68)
		<i>P</i> = .3*		<i>P</i> = .33*	
Conotruncal defects, n (%)	36 (57)	19 (48)	16 (84)	21 (62)	10 (45)
		<i>P</i> = .010		<i>P</i> = .36	

P values obtained by Wilcoxon rank-sum test for continuous variables or Pearson χ^2 test for categorical variables. *nNO*, Nasal nitric oxide. **P* value determined by the Fisher exact test.

TABLE E3. CM and nNO values in recruited CHD patients

ID #	Initial CM	Reciliation CM	Final CM	nNO, nL/min
7004	Abnormal		Abnormal	1.2
7027	Abnormal		Abnormal	Not available
7035	Not available		Not available	1.6
7040	Normal		Normal	1.6
7042	Abnormal		Abnormal	Not available
7053	Normal		Normal	1.9
7057	Not available		Not available	5.6
7058	Abnormal		Abnormal	62.8
7059	Not available		Not available	118.8
7102	Abnormal		Abnormal	87.6
7113	Abnormal	Abnormal	Abnormal	28.1
7129	Normal	Normal	Normal	Not available
7130	Normal		Normal	Not available
7134	Abnormal		Abnormal	Not available
7146	Normal	Normal	Normal	Not available
7151	Normal	Abnormal	Abnormal	Not available
7168	Normal	Normal	Normal	10.4
7190	Normal		Normal	10.3
7199	Normal		Normal	10.3
7208	Abnormal		Abnormal	9.4
7225	Normal	Normal	Normal	18.5
7242	Abnormal		Abnormal	16.1
7253	Abnormal		Abnormal	11.0
7266	Abnormal	Normal	Normal	17.4
7281	Abnormal		Abnormal	9.6
7288	Abnormal		Abnormal	14.6
7295	Abnormal	Abnormal	Abnormal	11.2
7302	Normal		Normal	5.8
7306	Abnormal		Abnormal	4.6
7319	Abnormal		Abnormal	16.7
7336	Normal	Normal	Normal	20.0
7351	Abnormal	Normal	Normal	4.6
7374	Normal	Not yet scored	Normal	13.4
7376	Normal		Normal	8.2
7379	Normal		Normal	11.3
7380	Normal		Normal	20.8
7381	Normal		Normal	12.0
7386	Normal		Normal	2.8
7389	Normal		Normal	9.7
7397	Normal		Normal	11.4
7400	Normal	Normal	Normal	9.3
7401	Normal	Normal	Normal	15.8
7405	Normal		Normal	29.9
7409	Abnormal	Abnormal	Abnormal	10.2

(Continued)

TABLE E3. Continued

ID #	Initial CM	Reciliation CM	Final CM	nNO, nL/min
7411	Normal	Normal	Normal	14.9
7414	Normal	Normal	Normal	12.1
7415	Normal	Normal	Normal	18.0
7421	Normal		Normal	14.6
7422	Normal		Normal	1.8
7425	Normal		Normal	16.7
7428	Abnormal		Abnormal	19.4
7433	Normal	Normal	Normal	12.1
7437	Normal		Normal	28.7
7438	Normal	Normal	Normal	12.2
7441	Abnormal	Abnormal	Abnormal	22.5
7444	Normal	Normal	Normal	18.9
7447	Normal	Normal	Normal	12.9
7448	Not available		Not available	19.3
7449	Normal		Normal	15.1
7450	Normal	Normal	Normal	15.3
7458	Normal	Normal	Normal	3.5
7459	Normal		Normal	8.0
7466	Normal		Normal	20.0

CM, Ciliary motion; nNO, nasal nitric oxide.

TABLE E4. Postoperative medication use subcategorized by CM and nNO

	Events with CM data (n = 91)		Events with nNO data (n = 83)	
	Normal (n = 58)	Abnormal (n = 33)	Normal (n = 48)	Low (n = 35)
Budesonide, n (%)	1 (1.7)	2 (6.1)	0 (0)	2 (6.0)
Cisatracurium, n (%)	44 (75.9)	22 (66.7)	32 (66.7)	27 (77.1)
Epinephrine, n (%)	55 (94.8)	31 (93.9)	45 (93.8)	33 (94.3)
Dexamethasone, n (%)	20 (34.5)	10 (30.3)	18 (37.5)	10 (28.6)
Heliox, n (%)	6 (10.3)	0 (0)	4 (8.3)	2 (5.7)
Hydrocortisone, n (%)	19 (32.8)	3 (9.1)	14 (29.2)	10 (28.6)
Inhaled nitric oxide, n (%)	20 (34.5)	4 (12.1)	13 (27.1)	10 (28.6)
Methylprednisolone, n (%)	5 (8.6)	1 (3.0)	3 (6.2)	3 (8.6)
N-Acetylcysteine, n (%)	2 (3.4)	4 (12.1)	2 (4.2)	1 (2.9)
Nitroprusside, n (%)	11 (19.0)	12 (36.4)	9 (18.8)	11 (31.4)
Racemic epinephrine, n (%)	8 (13.8)	1 (3.0)	4 (8.3)	5 (14.3)
Sildenafil, n (%)	1 (1.7)	1 (3.0)	2 (4.2)	0 (0)
Surfactant, n (%)	4 (6.9)	5 (15.2)	7 (14.6)	4 (11.4)

Data are median (interquartile range) or n (%). CM, Ciliary motion; nNO, nasal nitric oxide.

TABLE E5. Postsurgical outcomes subcategorized by CM and nNO

	Events with CM data (n = 91)		Events with nNO data (n = 83)	
	Normal (n = 58)	Abnormal (n = 33)	Normal (n = 48)	Low (n = 35)
CICU readmission, n (%)	7 (12.1)	3 (9.1)	3 (6.2)	5 (14.3)
Chest tube, d	6.5 (5-9)	6 (4-8)	6 (5-8)	6 (4.5-9.5)
Chest tube drainage, mL	626 (347-983)	486 (186-955)	606 (339-957)	486 (234-1041)
Diaphragm paralysis, n (%)	0 (0)	2 (6.1)	0 (37.5)	2 (5.7)
Fungal infection, n (%)	NA*	NA*	0 (0)	1 (2.9)
Intrinsic airway disease, n (%)	5 (8.8)	3 (9.1)	4 (8.5)	4 (11.4)
Nasogastric tube, n (%)†	31 (53.4)	20 (60.6)	27 (56.2)	21 (60.0)
Necrotizing enterocolitis, n (%)	4 (6.9)	3 (9.1)	4 (8.3)	3 (8.5)
Pleural effusion, n (%)	15 (25.9)	6 (18.2)	10 (20.8)	10 (28.6)
Pneumonia, n (%)	0 (0)	2 (6.1)	0 (0)	0 (0)
Pneumothorax, n (%)	1 (1.7)	3 (9.1)	2 (4.2)	0 (0)
Tracheitis, n (%)	1 (1.7)	2 (6.1)	0 (4.2)	2 (5.7)
Tracheostomy, n (%)	1 (1.7)	0 (0)	0 (0)	1 (4.5)

Data are median (interquartile range) or n (%). CM, Ciliary motion; nNO, nasal nitric oxide; CICU, cardiac intensive care unit; NA, not available. *NA = not available as the single fungal infection occurred in a patient who did not have a CM sample. †Need for nasogastric tube at time of hospital discharge to meet nutritional requirement.

TABLE E6. Detailed postoperative infection data for study participants with infection

ID#	Surgical encounter	Infection type	Organism	Site
7004	1	Bacterial	<i>Enterococcus faecalis</i>	Blood
7004	2	Bacterial	Coagulase-negative <i>Staphylococcus</i>	Blood
7027	1	Bacterial	Vancomycin-resistant <i>Enterococcus</i>	Urine
7035	1	Fungal	<i>Candida albicans</i>	Blood
7040	1	Bacterial	<i>Pseudomonas aeruginosa</i>	Urine
7042	1	Bacterial	<i>Stenotrophomonas maltophilia</i>	CSF
7058	1	Bacterial	<i>Enterococcus faecalis</i>	Mediastinum
7102	1	Bacterial	Culture negative	Mediastinum
7113	1	Bacterial	<i>Moraxella catarrhalis</i>	Trachea
7113	3	Viral	Rhinovirus/enterovirus	Nares
7134	1	Bacterial	<i>Streptococcus pneumoniae</i>	Blood
7134	1	Bacterial	<i>Proteus mirabilis</i>	Urine
7134	1	Viral	Rhinovirus/enterovirus	Trachea
7134	2	Bacterial	Coagulase-negative <i>Staphylococcus</i>	Mediastinum
7134	2	Viral	Respiratory syncytial virus	Nares
7168	1	Bacterial	Coagulase-negative <i>Staphylococcus</i>	Blood
7168	3	Bacterial	Coagulase-negative <i>Staphylococcus</i>	Mediastinum
7199	1	Bacterial	Culture negative	Blood
7242	1	Bacterial	Culture negative	Abdomen
7302	1	Bacterial	Culture negative	Mediastinum
7319	1	Bacterial	Coagulase-negative <i>Staphylococcus</i>	Blood
7351	2	Bacterial	<i>Klebsiella oxytoca</i>	Urine
7376	2	Bacterial	<i>Pseudomonas aeruginosa/Klebsiella oxytoca</i>	Trachea
7386	1	Bacterial	Culture negative	Mediastinum
7389	1	Bacterial	Culture negative	Abdomen
7401	1	Bacterial	Culture negative	Mediastinum
7430	1	Viral	Rhinovirus	Nares
7438	2	Bacterial	<i>Enterococcus faecalis</i>	Blood
7449	1	Bacterial	Culture negative	Abdomen
7450	1	Bacterial	Culture negative	Mediastinum
7466	1	Bacterial	Coagulase-negative <i>Staphylococcus</i>	Blood

CSF, Cerebrospinal fluid.