



CLINICAL RESEARCH ARTICLE

Differentiating esophageal sensitivity phenotypes using pH–impedance in intensive care unit infants referred for gastroesophageal reflux symptoms

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BACKGROUND: To identify esophageal sensitivity phenotypes relative to acid (S_{Acid}), bolus (S_{Bolus}), acid and bolus ($S_{\text{Acid+Bolus}}$), and none (S_{None}) exposures in infants suspected with gastroesophageal reflux disease (GERD).

METHODS: Symptomatic infants ($N = 279$) were evaluated for GERD at 42 (40–45) weeks postmenstrual age using 24-h pH–impedance. Symptom-associated probability (SAP) for acid and bolus components defined esophageal sensitivity: (1) S_{Acid} as $\text{SAP} \geq 95\%$ for acid ($\text{pH} < 4$), (2) S_{Bolus} as $\text{SAP} \geq 95\%$ for bolus, (3) $S_{\text{Acid+Bolus}}$ as $\text{SAP} \geq 95\%$ for acid and bolus, or (4) S_{None} as $\text{SAP} < 95\%$ for acid and bolus.

RESULTS: Esophageal sensitivity prevalence (S_{Acid} , S_{Bolus} , $S_{\text{Acid+Bolus}}$, S_{None}) was 28 (10%), 94 (34%), 65 (23%), and 92 (33%), respectively. Emesis occurred more in S_{Bolus} and $S_{\text{Acid+Bolus}}$ vs S_{None} ($p < 0.05$). Magnitude (#/day) of cough and emesis events increased with S_{Bolus} and $S_{\text{Acid+Bolus}}$ vs S_{None} ($p < 0.05$). $S_{\text{Acid+Bolus}}$ had increased acid exposure vs S_{None} ($p < 0.05$). Distributions of feeding and breathing methods were distinct in infants with S_{Bolus} vs S_{None} (both, $p < 0.05$). Multivariate analysis revealed that arching and irritability events/day were lesser at higher PMAs ($p < 0.001$) and greater for infants on NCPAP ($p < 0.01$) with S_{Bolus} and $S_{\text{Acid+Bolus}}$ ($p < 0.05$). Coughs/day was greater at higher PMAs ($p < 0.001$) for infants with gavage and transitional feeding methods ($p < 0.02$) with S_{Bolus} and $S_{\text{Acid+Bolus}}$ ($p < 0.05$) but lesser with Trach ($p < 0.001$). Number of emesis events/day were greater with S_{Bolus} and $S_{\text{Acid+Bolus}}$ ($p < 0.001$). Sneezes/day decreased for infants on Trach ($p = 0.02$).

CONCLUSIONS: Feeding and breathing methods can influence the frequency and type of aerodigestive symptoms. We differentiated esophageal sensitivity phenotypes in NICU infants referred for GERD symptoms using pH–impedance. Acid sensitivity alone was rare, which may explain poor response to acid suppressives; aerodigestive symptoms were predominantly linked with bolus spread. Magnitude of esophageal acid exposure and esophageal sensitivity to bolus spread may explain the pathophysiological basis for symptoms.

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IMPACT:

- Objective GERD diagnosis and reasons for symptoms in NICU infants remains unclear.
- Differentiation of esophageal sensitivities by acid and bolus components of GER reveal distinct symptom profiles, specifically the bolus component of GER significantly contributes to symptom occurrence.
- Acid only sensitivity to GER is rare, and acid-suppressive therapy alone may not improve symptoms in a majority of NICU infants.
- Magnitude of esophageal acid exposure and esophageal sensitivity to any bolus spread may explain the pathophysiological basis for symptoms. Feeding and breathing methods can influence the frequency and type of aerodigestive symptoms.
- GERD treatments should be individualized to the patient's GERD phenotype and likely also target the bolus component of GER.

INTRODUCTION

Gastroesophageal reflux (GER) is defined as the passage of gastric contents into the esophagus with or without regurgitation; GER disease (GERD) is when reflux is associated with troublesome symptoms.^{1–3} GERD diagnosis rates vary, ranging from 2% to 30% among US neonatal intensive care units (NICUs), amounting to an

additional burden of >\$70,000 per admission.⁴ Ambiguity lies with the definition of troublesome symptoms in neonates or non-verbal patients in general. As there is no established gold standard for clarifying the basis of symptoms,² it is difficult to prove objectively if and which symptoms are truly due to GERD. As a result, infants are frequently subjected to overprescription and

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prolonged use of acid-suppressive therapies,^{4–7} modified nutrition and feeding strategies,^{8,9} missed feeding opportunities,¹⁰ and increased prevalence of procedures, including gastrostomy and fundoplication.^{11,12} It is well accepted that symptom relief in infants may not happen despite the acid-suppressive therapies.^{3,6,7} Previous research has demonstrated phenotyping of GERD based on pH-impedance monitoring, which can benefit the decision-making process with management.¹³ Symptoms may occur due to the esophageal sensitivity to acid, bolus, both (acid+bolus), or due to other non-GER causes and can be investigated using 24-h pH-impedance with symptom correlation metrics.^{3,14–18}

By identifying these phenotypes, targeted therapeutic strategies can be developed, so that unnecessary therapies can be avoided. Thus our rationale was that symptom association with GER events can be clarified using phenotype classification based on pH-impedance characteristics.^{14,17,18}

Our aim was to define and distinguish the esophageal sensitivity to acid (S_{Acid}), bolus (S_{Bolus}), acid+bolus ($S_{\text{Acid+Bolus}}$), or none (S_{None}) in relation to symptom occurrence. We hypothesized that symptom occurrence is related to esophageal sensitivity phenotypes.

METHODS

Participants and setting

Data from consecutive pH-impedance studies was retrospectively analyzed from NICU infants ($N = 279$) referred to the Innovative Neonatal and Infant Feeding Disorders Research Program at Nationwide Children's Hospital (Columbus, OH, USA) for the evaluation of GERD. Inclusion criteria included infants who (1) had 24-h pH-impedance testing between 2012–2015 with ≥ 18 h of analyzable pH-impedance data and (2) were enterally fed at the time of evaluation and (3) were < 60 weeks postmenstrual age (PMA) at the time of evaluation. Exclusion criteria included infants who received treatment with acid-suppressive therapy either prior to or during evaluation and those on continuous gavage feeds. Institutional Review Board approval was obtained, and Health Insurance Portability and Accountability Act guidelines were followed. Informed parental consent was obtained prior to pH-impedance testing.

Experimental protocol

Subjects underwent 24-h pH-impedance testing using a disposable pH-impedance probe with six impedance channels and one distal pH sensor (Greenfield MMS-Z1-I or ZandorPH MMS-Z1-P-7R, Laborie Medical Technologies, Mississauga, ON, Canada) attached to a recording device (MMS Ohmega, Laborie Medical Technologies, Mississauga, ON, Canada) as previously published.^{14,16–20} The probe was calibrated using buffer solutions with pH 4.0 and pH 7.0. The catheter was positioned using estimation equations²¹ and verified by chest X-ray so that the pH sensor was located between T7 and T8 vertebrae.^{18,19} Per testing protocol, studies were performed in supine position. Any events or symptoms documented during meal times or infant cares were not analyzed.

Data analysis

Clinical characteristics. Reasons for GERD testing were grouped into dysphagia-related concerns (poor oral feeding, oral aversion, feeding intolerance, failure to thrive, or choking during feeds), GER-type symptoms (arching/irritability or emesis), airway-related concerns (cough, stridor, suspected or confirmed aspiration, or persistent/escalating oxygen requirement needs), or cardiopulmonary concerns (apnea/bradycardia/desaturation). An infant may have had more than one concern. Feeding characteristics, including total fluid volume, mL/kg/day; feeding type (exclusive breast milk:exclusive formula:mixed), %; and feeding frequency per subject per day were analyzed.

pH-impedance metrics. Data were analyzed using the MMS analysis software (v. 9.5, Laborie Medical Technologies, Mississauga, ON, Canada). Acid and bolus GER components were evaluated as previously published.^{14,16–20,22–25} Briefly, acid GER was defined as events with pH < 4 for > 5 s duration. Bolus GER events were defined as retrograde movement of bolus marker evidenced by 50% drop in impedance, originating in the Z6 channel and reaching at least one channel above.^{14,22} In addition, we analyzed acid reflux index (ARI) as the percentage of time esophagus was exposed to acid, which was further categorized based on ARI severity into normal (ARI $< 3\%$), indeterminate (ARI 3–7%), or abnormal (ARI $> 7\%$) as per published guidelines,¹ and number of reflux events (acid+non-acid) > 100 /day.²⁰ Detailed characteristics of acid exposure events per day were examined for the number of events > 5 min/day, the longest duration, pH only events, and acid clearance time.^{23,24} Impedance characteristics^{14,17–19,22} were analyzed for: (a) total number (per day) for any ascending events, liquid, gas, mixed, acid (pH < 4), weakly acid (pH 4–7), and weakly alkaline (pH > 7) events, (b) bolus clearance time, s, and (c) distal baseline impedance, ohms, categorized by severity (< 900 , 900–2000, > 2000).

Symptoms. Symptoms were identified and documented in real time by trained nurses or nurse assistants blinded to the study recordings. They were present at the bedside in 6–8 h shifts, continuously for 24 h for each patient. As previously published,^{14,16–18,26} commonly reported symptoms included: arching/irritability defined as back arching with head and neck extension accompanied by irritability or crying, audibly detected cough, sneeze, hiccoughs, or stridor, visually observed emesis, grimacing, gagging or flushing, and apnea/bradycardia/desaturation defined as a pause in breathing > 20 s, heart rate < 80 bpm ≥ 10 s, or oxygen saturation $< 80\%$ ≥ 10 s, respectively.²⁷ Individual symptom prevalence was counted if a symptom occurred at least once during the 24-h study duration.

Definition of esophageal sensitivities due to GER events. Symptom-associated probability (SAP) is defined as the statistical relationship between symptoms and reflux episodes calculated by Fisher's exact test, with SAP $\geq 95\%$ indicating that the observed associations did not occur by chance, thus were likely caused by GER.^{14,28,29} SAP values were calculated using 2-min windows; reflux was considered symptomatic when a reflux event occurred within 2-min before the onset of the symptom. To determine the GERD phenotype for each subject, SAP was calculated for both acid (pH sensor) and bolus (impedance sensors) components of GER (Fig. 1). To clarify, (1) Sensitivity to acid only (S_{Acid}) was defined as pH SAP $\geq 95\%$ and impedance SAP $< 95\%$, (2) Sensitivity to bolus only (S_{Bolus}) was defined as having pH SAP $< 95\%$ and impedance SAP $\geq 95\%$, (3) Sensitivity to acid and bolus ($S_{\text{Acid+Bolus}}$) was defined as pH SAP $\geq 95\%$ and impedance SAP $\geq 95\%$, and (4) No sensitivity (S_{None}) was defined as pH SAP $< 95\%$ and impedance SAP $< 95\%$.

Statistical analysis. Kruskal–Wallis and Fisher's exact tests were used to compare demographic characteristics, outcomes, prevalence, and distribution of symptoms between groups.²⁸ Dunn's multiple comparison test was used to perform multiple pairwise comparisons. pH-impedance characteristics were compared using Welch's analysis of variance with Tukey and t tests for multiple comparisons. Multivariate models were constructed to examine the association of PMA, feeding method at evaluation, breathing method at evaluation, GERD phenotypes, bronchopulmonary dysplasia (BPD), and neuropathology with the number of arching/irritability, coughs, emesis, and sneezes per day. Partial F tests were used to examine the significance of each variable in the model. Bonferroni adjustments were applied for all multiple comparisons. Effects of BPD and neuropathology were also

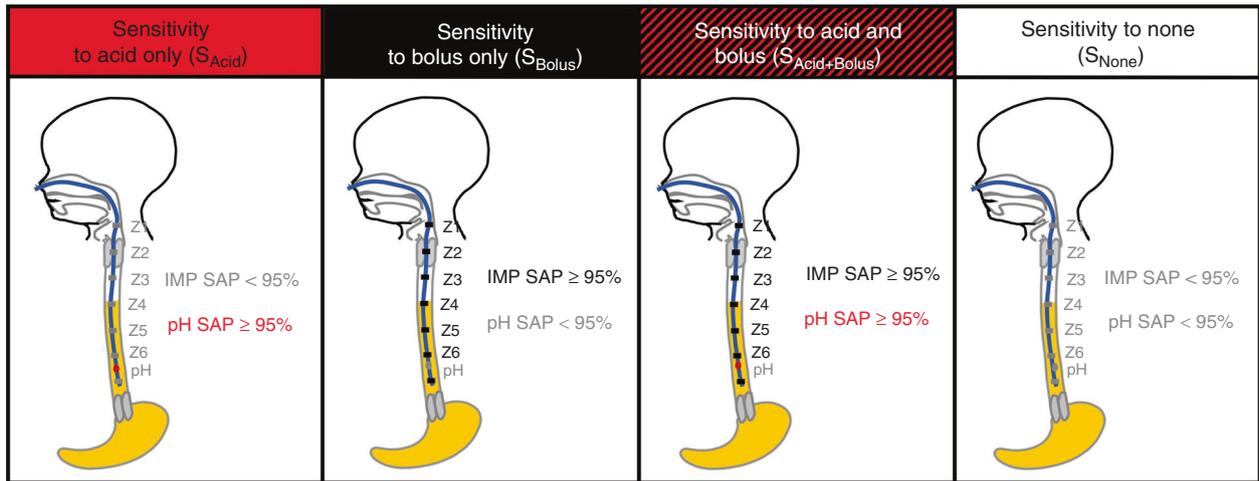


Fig. 1 Classification of the esophageal sensitivity groups. SAP $\geq 95\%$ for acid events (as detected by pH) was classified as an acid sensitivity, and SAP $\geq 95\%$ for bolus events (as detected by impedance) was classified as a bolus sensitivity. A patient was grouped as having (1) S_{Acid} , (2) S_{Bolus} , (3) $S_{Acid+Bolus}$, or (4) S_{None} (SAP $< 95\%$ for acid and bolus). SAP $\geq 95\%$ = abnormal. Note that the four color coding represents esophageal sensitivity groups: Red = sensitivity to acid (S_{Acid}); black = sensitivity to bolus (S_{Bolus}); red and black = sensitivity to acid and bolus ($S_{Acid+Bolus}$); white = sensitivity to none (S_{None}).

analyzed. p Values < 0.05 were considered statistically significant. Data are presented as median (interquartile range (IQR)), mean \pm SD, $\beta \pm$ SE, or %.

RESULTS

Clinical outcomes

Overall, 279 infants (50 males) born at median of 28.7 weeks' (IQR 26.1–33.6 weeks) gestation and evaluated at a median PMA of 42.4 weeks (IQR, 40.3–45.1) were evaluated from 6995 h of pH–impedance data. APGAR scores, median (IQR) were 4 (1–9) at 1 min and 7 (1–10) at 5 min. Perinatal neuropathology was present in 102 (37%), of which 18 (18%) were hypoxic ischemic neuropathology, 66 (65%) were intraventricular hemorrhage, and 18 (18%) were parenchymal changes (gray and white matter). Congenital anomalies were present in 49 (18%), of which were genetic in 22 (45%), neurologic in 9 (18%), airway in 2 (4%), gastrointestinal in 10 (20%), renal in 3 (6%), and miscellaneous in 3 (6%). Reasons for GERD testing were: (a) dysphagia-related concerns in 121 (43%), (b) GER-type symptoms in 106 (38%), (c) airway-related concerns in 21 (8%), and (d) cardiopulmonary concerns in 107 (38%). Prevalence of symptom presence among the 279 infants was 100% for arching/irritability, 98.9% for coughing, 86.4% for sneezing, 73.8% for grunting, 51.6% for emesis, 40.1% for hiccough, 38.4% for apnea/bradycardia/desaturation, 36.9% for gagging, 35.5% for grimace, 20.8% for yawning, 11.5% for flushing, and 4.3% for stridor. Symptom occurrence per patient (#/day) was 56 (35–80) for arching/irritability, 12 (7–22) for cough, 4 (1–8) for sneeze, 6 (0–23) for grunt, 1 (0–2) for emesis, 0 (0–1) for hiccough, apnea/bradycardia/desaturation, and gag, 0 (0–2) for grimace, and 0 (0–0) for yawn, flush, and stridor. In infants with neuropathology ($N = 102$) vs no neuropathology ($N = 177$), total symptom occurrence (127 \pm 64 vs 144 \pm 68 symptoms/day respectively, $p = 0.1$) and specific symptom occurrences (all $p > 0.05$ for arching/irritability, cough, grunt, sneeze, emesis, hiccough, and apnea/bradycardia/desaturation) did not significantly differ. In infants with BPD ($N = 155$) vs no BPD ($N = 124$), symptom occurrence was increased (148 \pm 72 vs 126 \pm 60 symptoms/day, respectively, $p < 0.01$), specifically for arching/irritability with 76 (49–106) vs 62 (34–90) per day, respectively, $p = 0.02$, while sneezing was significantly decreased with 4 (2–8) vs 7 (4–11) per day, respectively, $p = 0.01$. Other specific symptoms were not significant (all $p > 0.5$ for cough, grunt, emesis, hiccough, and

apnea/bradycardia/desaturation. The prevalence of infants with ARI $> 7\%$ was 38%, and reflux events > 100 was 18%.

Esophageal sensitivity groups

Demographics and prevalence of esophageal sensitivity groups are shown (Table 1, Fig. 2). Majority of demographic and outcome characteristics did not significantly differ between the esophageal sensitivity groups ($p > 0.05$). The pH–impedance characteristics of the esophageal sensitivity groups are shown (Table 2).

The prevalence and magnitude of symptoms by esophageal sensitivity groups are shown (Fig. 3). Emesis prevalence was significantly greater with S_{Bolus} and $S_{Acid+Bolus}$ ($p < 0.05$ vs S_{None} , Fig. 3a). Frequency of the following symptoms was increased (vs S_{None}): cough for S_{Bolus} and $S_{Acid+Bolus}$, sneezing for S_{Bolus} , and emesis for S_{Bolus} and $S_{Acid+Bolus}$ (all $p < 0.05$, Fig. 3b). ARI severity among the esophageal sensitivity groups is shown in Fig. 4.

Comparing proportions of esophageal sensitivity ($S_{Acid} : S_{Bolus} : S_{Acid+Bolus} : S_{None}$) for: (1) ARI $\leq 7\%$ ($N = 173$) vs ARI $> 7\%$ ($N = 106$) was 9:35:17:39 vs 11:31:35:23, respectively, $p = 0.001$, (2) reflux events ≤ 100 ($N = 184$) vs reflux events > 100 ($N = 38$) were 0:40:10:50 vs 10:34:24:32, respectively, $p = 0.4$, (3) neuropathology ($N = 102$) vs no neuropathology ($N = 177$) were 13:33:17:37 vs 9:34:27:30, respectively, $p = 0.2$, and (4) BPD ($N = 155$) vs no BPD ($N = 124$) was 10:33:24:33 vs 10:35:22:33, $p = 0.9$.

Multivariate models to examine the effect of modifying factors contributing to heterogeneity on key symptoms

Table 3 presents a summary of the significance of each variable and multivariate model fit statistics, and Table 4 summarizes the multivariate models which include all variables that could be tested in this study. We note that the number of arching and irritability events per day was lesser at higher PMAs ($\beta \pm$ SE, -0.13 ± 0.03 , $p < 0.001$) but greater for infants on nasal continuous positive airway pressure (NCPAP) ($N = 13$, 2.13 ± 0.85 , $p < 0.01$) and greater for S_{Bolus} (0.77 ± 0.36) and $S_{Acid+Bolus}$ (0.96 ± 0.39) infants ($p < 0.05$). Number of coughs per day was greater at higher PMA (0.08 ± 0.02 , $p < 0.001$), greater for infants on tube (1.05 ± 0.45) and transitional (1.21 ± 0.24) feeding methods ($p < 0.02$), greater for S_{Bolus} (0.7 ± 0.27) and $S_{Acid+Bolus}$ (0.63 ± 0.3) infants ($p < 0.05$) but lesser for infants on tracheostomy ($N = 10$, -2.29 ± 0.7 , $p < 0.001$). Number of emesis events per day is increased for S_{Bolus} (0.51 ± 0.15) and $S_{Acid+Bolus}$ (0.56 ± 0.16) infants ($p < 0.001$). Number of sneezes per day is decreased for infants on tracheostomy (-1.12

Table 1. Clinical and outcome characteristics distributed by the esophageal sensitivity groups.

Characteristics	Overall	Sensitivity group				p Value
		S _{Acid} (N = 28)	S _{Bolus} (N = 94)	S _{Acid+Bolus} (N = 65)	S _{None} (N = 92)	
At birth						
Gestational age, weeks	29 (26–34)	28 (25–31)	28 (26–33)	29 (26–33)	30 (26–34)	0.5
Premature birth (≤37.0 weeks), %	89	89	88	92	86	0.7
Gender, male, %	50	50	50	52	48	1
At evaluation						
Postmenstrual age, weeks	42 (40–45)	43 (40–45)	43 (41–46)	42 (41–45)	42 (40–45)	0.9
Chronologic age, weeks	13 (9–17)	13 (9–17)	13 (10–18)	14 (9–17)	12 (8–16)	0.4
Weight at evaluation, kg	3.6 (3.1–4.4)	3.8 (3.0–4.2)	3.5 (3.1–4.4)	3.7 (3.2–4.4)	3.5 (3.0–4.4)	0.8
Bronchopulmonary dysplasia, %	56	57	54	57	55	1
Neuropathology, %	36	43	36	26	41	0.2
Feeding method (tube:transitional:oral), %	15:47:38	11:61:28	5:51:44*	14:46:40	28:39:33	<0.01
Breathing method (trach:NCPAP:NC:none), %	4:5:43:48	4:0:52:44	1:1:49:49*	1:5:39:55	8:10:39:43	0.03
At discharge						
Length of hospital stay, days	81 (32–140)	81 (35–143)	86 (34–139)	83 (35–133)	71 (31–149)	0.8
Feeding method (tube:transitional:oral), %	13:27:60	18:18:64	6:31:63	17:24:59	15:29:56	0.3
Breathing method (trach:NC:none), %	4:38:58	0:46:54	0:38:62	5:36:59	8:37:55	0.1
Fundoplication, %	2	4	1	2	4	0.4

Data presented as median (IQR) or %.
trach tracheostomy, NCPAP nasal continuous positive airway pressure, NC nasal cannula.
*p < 0.05 vs no sensitivity group.

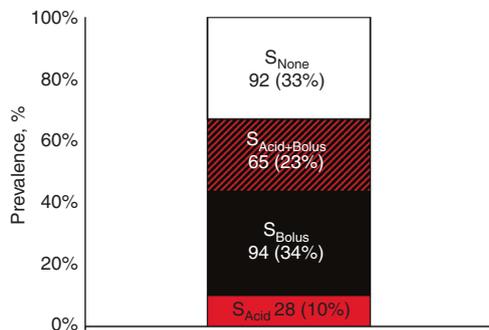


Fig. 2 Prevalence of esophageal sensitivity categories. S_{Acid} was observed in only 10% of infants, sensitivity to any acid (S_{Acid}+S_{Acid+Bolus}) GER was 33%. Sensitivity to any bolus (S_{Bolus}+S_{Acid+Bolus}) GER was 57%, and prevalence of symptoms due to acid and/or bolus GER (S_{Acid}+S_{Bolus}+S_{Acid+Bolus}) was 67%.

± 0.49, p = 0.02). Note that BPD and neuropathology did not have a significant relationship with any of the symptoms in the presence of other predictors.

DISCUSSION

Little is known as to why symptoms are attributed to GERD in NICU infants. The value of pH-impedance testing in the classification of GERD phenotypes in NICU infants, or in any pediatric age groups, has not been established. We have undertaken this study to delineate potential mechanisms for the troublesome symptoms linked with GERD in infants where symptom interpretation can be challenging, while acid-suppressive therapies, feeding diversion strategies, gastrostomy, and fundoplication procedures are widely prevalent. We categorized esophageal sensitivity based on SAP correlation with acid (as detected by the pH sensor) and bolus (as detected by impedance channels) GER components.

The most noteworthy feature of our study underlies in the unique methodological approach we have undertaken to classify the GERD phenotypes from a large cohort of infants at their first evaluation for GERD. Our approaches could be applied in any non-verbal patient situations so as to characterize the GERD phenotypes with accuracy. Four phenotypes of esophageal sensitivity based on SAP ≥ 95% to acid (S_{Acid}), bolus (S_{Bolus}), acid + bolus (S_{Acid+Bolus}), or none (S_{None}) were distinguished. Given that the demographic comparisons are similar across the four phenotypes, the salient findings are as follows: (1) Esophageal sensitivities: Prevalence of S_{Acid} is rare, with S_{Bolus} or S_{Acid+Bolus} being more common. These categories may serve as potential therapeutic targets and define indications for therapies. (2) Symptoms: All infants experience arching/irritability and cough, which were the dominant symptoms. However, the number of coughs and emesis were higher in those with sensitivity to bolus components. These diagnostic thresholds may be useful in infants referred for GERD suspicion, which need further objective verification. (3) pH-impedance characteristics: Those with bolus only, or acid and bolus spread, may present similarly. The acid exposure duration was similar across all phenotype groups. (4) ARI characteristics specific for the number of acid reflux events and those >5 min, longest acid reflux events, and distal esophageal exposure were all increased in those with S_{Acid+Bolus}, compared to S_{None}. In addition, when evaluating abnormal ARI (>7%) vs ARI ≤ 7%, abnormal ARI had increased esophageal sensitivity. However, abnormal frequency of esophageal events (>100 events/day) did not impact esophageal sensitivity. Also note, some of those with normal ARI exhibited sensitivity to GER and conversely some of those with minimal symptoms had abnormal ARI. Thus acid-suppressive therapies to decrease gastric acidity may not be the solution for all patients with reflux type of symptoms. (5) We noted that BPD and neuropathology did not have a significant relationship with any of the symptoms in the presence of other predictors. Feeding and breathing methods can influence the frequency and type of aerodigestive symptoms. (6) Multivariate analysis revealed that arching and irritability events/day were

Table 2. pH-impedance and feeding regimen characteristics by the esophageal sensitivity groups.

Characteristics	Overall	Sensitivity group				p Value
		S _{Acid} (N = 28)	S _{Bolus} (N = 94)	S _{Acid+Bolus} (N = 65)	S _{None} (N = 92)	
pH sensor						
Acid reflux index, %	7.2 ± 7.2	8.2 ± 7.3	7.4 ± 8.7	9.6 ± 7.6*	5.2 ± 5.6	<0.01
ARI category (normal:indeterminate:abnormal), %	36:26:38	25:32:43	35:30:35	23:20:57*	49:25:26	<0.01
Acid reflux events, #/day	67 ± 55	84 ± 60	65 ± 56	86 ± 57*	49 ± 45	<0.01
Acid reflux events >5 min, #	4 ± 5	4 ± 5	5 ± 6	6 ± 5*	3 ± 5	0.01
Longest acid reflux, min	15 ± 16	16 ± 17	15 ± 14	20 ± 17*	11 ± 16	<0.01
pH only events, #	49 ± 42	63 ± 42	45 ± 41	62 ± 46*	45 ± 41	<0.01
Acid clearance time, s	108 ± 263	107 ± 14	105 ± 8	117 ± 9	104 ± 9	0.7
Impedance sensors						
Total ascending reflux events	64 ± 39	55 ± 28	76 ± 41*	70 ± 41	48 ± 34	<0.01
Liquid events, #/day	60 ± 45	52 ± 33	74 ± 48*	70 ± 48*	41 ± 35	<0.01
Gas events, #/day	6 ± 12	8 ± 18	4 ± 8	5 ± 7	7 ± 16	0.4
Mixed events, #/day	23 ± 23	23 ± 16	26 ± 26	30 ± 25*	16 ± 18	0.01
Gas events, #/day	6 ± 12	8 ± 18	4 ± 8	5 ± 7	7 ± 16	0.4
Acid events, #/day	19 ± 18	19 ± 14	20 ± 20*	25 ± 19*	12 ± 13	<0.01
Weakly acid events, #/day	27 ± 20	27 ± 19	30 ± 21*	35 ± 20*	21 ± 18	<0.01
Weakly alkaline events, #/day	3 ± 9	0.9 ± 4	3 ± 11	2 ± 6	3 ± 11	0.5
Bolus clearance time, s	16 ± 16	16 ± 1	15 ± 1	16 ± 1	15 ± 1	0.9
DBI, ohms	1798 ± 605	1750 ± 653	1861 ± 632	1752 ± 717	1909 ± 540	0.4
DBI (<900:900–2000:>2000), %	6:56:38	11:54:35	5:55:40	11:57:32	2:59:39	0.3
Feeding regimen characteristics						
Total fluid volume, mL/kg/day	138 (126–150)	140 (129–150)	138 (125–150)	136 (125–150)	140 (126–150)	0.6
Feeding type (exclusive breast milk:exclusive formula:mixed), %	3:66:31	3:61:36	3:66:31	1:69:30	4:64:32	0.9
Feeding frequency, #/day	8 (7–8)	8 (7–9)	8 (7–8)	8 (7–8)	8 (7–8)	0.2

Data presented as mean ± SD, % or median (IQR) and BCT as mean ± SE.
*p < 0.05 vs no sensitivity group.

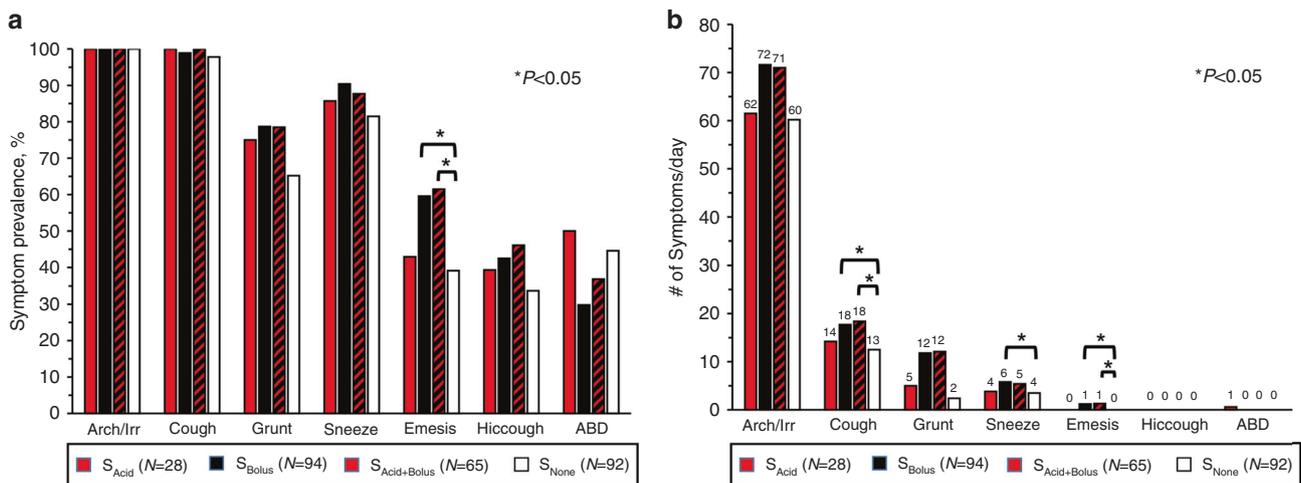


Fig. 3 Symptom prevalence and type in relation to esophageal sensitivity categories. **a** Symptom prevalence (%). Nearly all infants exhibited arching/irritability (100%) and cough (98.9%). Emesis was increased in infants with any bolus sensitivity vs no sensitivity. **b** Symptom magnitude (# symptoms/day). Arching/irritability symptoms occurred over 60 times per day but not significantly different between groups. Cough and emesis magnitude was significantly higher in infants with any bolus sensitivity vs no sensitivity. Sneezing magnitude was increased in bolus only sensitivity group vs no sensitivity. Grunting, hiccoughs, and apnea/bradycardia/desaturation events were infrequent among all groups.

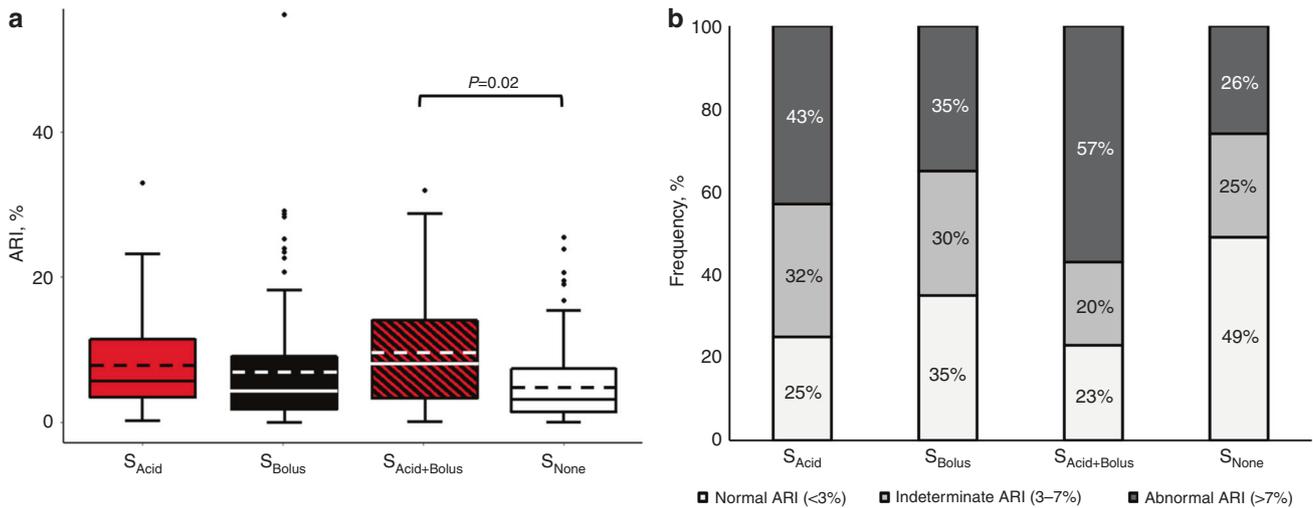


Fig. 4 Distribution of acid reflux index. Distribution of acid reflux index (ARI) severity as a continuous variable (a) and as categorical variable (b) across the esophageal sensitivity categories. a ARI was increased in S_{Acid+Bolus} sensitivity. b ARI distributions did not significantly differ between groups. SAP values $\geq 95\%$ were observed in even normal ARI groups. However, clinically 57% of those with S_{Acid+Bolus} have abnormal ARI.

Table 3. Significance of multivariate regression analysis variables on the number of symptoms per day.

Variables	$\sqrt{\text{Arching/irritability per day}}$	$\sqrt{\text{Coughs per day}}$	$\sqrt{\text{Emesis per day}}$	$\sqrt{\text{Sneezes per day}}$
PMA at evaluation	<0.001**	<0.001**	0.95	0.88
Feeding method at evaluation	0.61	<0.001**	0.70	0.66
Breathing method at evaluation	<0.01*	<0.01*	0.09	0.01*
GERD phenotypes	0.06	0.06	<0.001**	0.23
Neuropathology	0.45	0.73	0.20	0.21
BPD	0.05	0.66	0.69	0.18
Overall model significance	<0.001**	<0.001**	<0.001**	<0.001**
Adjusted R square	0.14	0.18	0.09	0.08

Table presents significance of explanatory power of variables on the square root transformation of number of symptoms per day from partial F tests, overall model significance, and their adjusted R squares.

* $p < 0.05$, ** $p < 0.001$ on outcome.

lesser at higher PMAs and greater for infants on NCPAP and for S_{Bolus} and S_{Acid+Bolus}. Number of coughs/day was greater at higher PMA, greater with gavage and transitional feeding methods compared to oral feeding methods, greater for S_{Bolus} and S_{Acid+Bolus}, but lesser in infants with tracheostomy. Number of emesis events/day were greater for S_{Bolus} and S_{Acid+Bolus}, and number of sneezes/day decreased in infants with tracheostomy.

The pathophysiological basis for our findings and symptoms can be explained as follows

In children and adults, GERD can present with acute or chronic symptoms.^{14,16,30,31} The symptoms can manifest as esophageal, supra-esophageal, or extra-esophageal and include heart burn, coughing and choking as in laryngeal penetration and aspiration, swallowing difficulties, sinusitis, pulmonary parenchymal disease, bronchospasm, and aggravation of asthma.^{32,33} The chronicity and severity of these symptoms may depend on the integrity of esophageal mucosal barrier,¹⁹ presence of esophageal or airway inflammation,^{34,35} pharyngo-esophageal motility,¹⁵ upper esophageal sphincter (UES) and lower esophageal sphincter (LES) function,^{36,37} underlying reserve or ability to recover from prolonged events, and central neurocognitive abilities. However, in infants or non-verbal patients (regardless of age), owing to the lack of objectivity and/or misinterpretation of cues as troublesome

symptoms attributed to GERD results in the use of modified nutrition and feeding strategies,^{8,9} missed feeding opportunities,¹⁰ acid-suppressive therapies,⁵⁻⁷ rise in gastrostomy procedures, and funduplications^{11,12} in infants.

Potential mechanisms for GER-associated clinical presentations and outcomes in our study are explained below and are based on several theoretical frameworks

(1) Reflux vs reflex theory: Reflux theory involves laryngeal stimulation-induced symptoms due to the retrograde flow of gastric contents into the larynx.^{30,38} Presentation of symptoms may include aspiration pneumonia, chronic cough, stridor, or bronchospasm. As only 4% of the population had tracheostomy and 2% underwent fundoplication, this mechanism is likely rare and should be considered in the context of 56% of the cohort as having BPD and 36% had neuropathology. However, it is plausible that this "reflux theory" mechanism may be the basis for disease chronicity and prolonged hospitalization in the NICU. Reflex theory involves distal esophageal or supra-esophageal refluxate triggering cranial (V, VII, IX, X, XI, XII) nerve-mediated reflexes and symptoms. These reflexes can be (a) locally mediated, i.e., within the contiguous esophageal column, secondary peristalsis reflex,^{39,40} LES relaxation reflex,⁴¹ UES contractile reflex,^{42,43} and may present with arching and irritability depending on UES

Table 4. Summary of multivariate regression analysis for predicting the number of symptoms per day.

Variables	√Arching/irritability per day			√Coughs per day			√Emesis per day			√Sneeze per day		
	β	SE	Sig	β	SE	Sig	β	SE	Sig	β	SE	Sig
Intercept	12.84	1.08	<0.001**	-0.16	0.83	0.85	0.88	0.45	0.05	2.50	0.58	0.00
PMA at evaluation	-0.13	0.03	<0.001**	0.08	0.02	<0.001**	0.00	0.01	0.95	0.00	0.01	0.88
Feeding method at evaluation—transitional	0.02	0.32	0.94	1.21	0.24	<0.001**	-0.08	0.13	0.56	-0.10	0.17	0.57
Feeding method at evaluation—tube	-0.53	0.59	0.37	1.05	0.45	0.02*	-0.19	0.24	0.43	-0.27	0.32	0.39
Breathing method at evaluation—NC	0.49	0.39	0.21	-0.17	0.30	0.58	-0.20	0.16	0.22	-0.08	0.21	0.69
Breathing method at evaluation—NCPAP	2.13	0.85	0.01*	-1.07	0.65	0.10	-0.33	0.35	0.35	-0.33	0.46	0.47
Breathing method at evaluation—trach	0.52	0.91	0.57	-2.29	0.70	<0.001**	-0.29	0.38	0.45	-1.12	0.49	0.02*
GERD phenotype—S _{Acid}	0.46	0.51	0.37	0.49	0.39	0.22	0.09	0.21	0.67	-0.01	0.28	0.98
GERD phenotype—S _{Acid+Bolus}	0.96	0.39	0.01*	0.63	0.30	0.04*	0.56	0.16	<0.001**	0.31	0.21	0.14
GERD phenotype—S _{Bolus}	0.77	0.36	0.03*	0.70	0.27	0.01*	0.51	0.15	<0.001**	0.34	0.19	0.08
Neuropathology	-0.24	0.31	0.45	0.08	0.24	0.73	-0.16	0.13	0.20	-0.21	0.17	0.21
BPD	0.74	0.37	0.05	0.13	0.29	0.66	-0.06	0.16	0.69	-0.27	0.20	0.18

p* < 0.05, *p* < 0.001 on square root transformation of the outcomes (Arching/irritability per day, coughs per day, emesis per day, and sneeze per day). Infant who was orally feeding, on room air, belonged to S_{None} phenotype, and did not have BPD or neuropathology at evaluation was the reference. Single asterisks (*) and double asterisks (**) indicate that the predictor had a significantly different effect on the outcome than the intercept, given that all other predictors remain unchanged, i.e., an infant who was tube fed, on room air, belonged to S_{None} phenotype, and did not have BPD or neuropathology had a significantly higher number of coughs than an infant who was orally feeding, on room air, belonged to S_{None} phenotype, and did not have BPD or neuropathology at evaluation.

involvement and arousals,⁴⁴ (b) supra-esophageal mediated, i.e., pharyngeal swallowing,^{39,45} and (c) extra-esophageal mediated, i.e., airway reflexes, laryngeal chemoreflex, glottal closure reflexes, stridor, and bronchospasm.⁴³ It is likely that the cues presented characterize protective reflex mechanisms and may not be truly “troublesome.” These entities may be suggestive of reflux-hypersensitivity that involves both airway reflexes and digestive reflexes.¹⁶ (2) Role of esophageal acid clearance and bolus clearance: the 24-h ARI can be a yardstick for the severity of acid exposure and may be a function of acid production, acid neutralization, mucosal integrity, esophageal motility, bolus clearance mechanisms, swallowing skills, and comorbidities. Bolus clearance time was similar in all four phenotypes of esophageal sensitivity, which suggests that the compensatory mechanisms are at play. However, frequency of GER events were variable and determined by GER causal mechanisms, of which transient LES relaxation is the most frequent, although hypotonic LES and gastroparesis are other possible causes. Our findings suggest that the bolus component of GER may be contributory to symptom occurrence. Nearly all infants had arching/irritability and coughing; however, only those with esophageal sensitivity to bolus had frequent cough, sneezing, and emesis. (3) Non-GERD mechanisms may be responsible for symptom generation: controversy still exists regarding the association of GER and apnea/bradycardia/desaturation events.^{46,47} In the current study, 38% of the infants were referred for cardiopulmonary concerns. However, the actual number of apnea/bradycardia/desaturation events reported were only 0 (0–1) events/day, which were not associated with any of the esophageal sensitivity profiles. We and others have shown that these events are rarely associated with GER mechanisms and is more likely due to dysfunctional swallowing, which may be modified by maturation and stimulus volume.^{15,46,48,49} Interestingly, 43% of GERD testing referrals were for dysphagia concerns. Thus we hypothesize that clinical presentation of GERD symptoms and eating/swallowing difficulties are likely co-dependent. Esophageal and/or pharyngeal provocation such as during ascending GER events can happen along with airway responses, as a consequence of bolus presence. Several reflexes can be triggered and are associated with symptoms and protective and clearance

responses. These include esophago-glottal closure reflex,⁴³ pharyngo-glottal closure reflex,⁵⁰ cough reflex,²⁶ pharyngeal reflexive swallowing,³⁹ laryngeal chemo reflex,⁵¹ esophago-deglutition reflex,⁴² secondary peristalsis,⁵¹ UES contractile reflex,⁵² and LES responses.^{41,45} Individual reflexes or combinations of these reflexes are responsible for airway protection and clearance and may be responsible for symptoms. When acute or chronic airway and digestive problems are noted and GERD is suspected, careful examination of structure–function is indicated. Both esophageal and swallowing/aerodigestive provocation can happen together (as evidenced by symptoms acutely and manifesting chronically as feeding difficulties), and the originating responses and clearance mechanisms are protective; when these are compromised, it requires step-up evaluations. (4) Influence of breathing methods on esophageal sensitivity: as depicted in Table 1, there was a higher proportion of infants on nasal cannula and room air in infants with S_{Bolus} vs S_{None}. Previously, we have shown in infants with chronic lung disease that esophageal sensitivity was high (as measured by symptom sensitivity index) with reflux events migrating to the pharynx.¹⁸ In that study, a majority of those infants were on nasal cannula oxygen. Results from another study of newborn lambs show that NCPAP may actually decrease the number of GER events.⁵³ This concept is also supported by another study in which we have shown that LES relaxation (a common mechanism of GER) is less frequent in infants on NCPAP,⁵⁴ thus may be the reason for decreased sensitivity to bolus.

The clinical implications are several

We note that sensitivity to acid alone is minimal and prolonged treatment with acid-suppressive medicines^{1,55} are not indicated. Patients treated with acid-suppressive medicines are frequently exposed to prolonged treatment.^{1,55} The number of symptoms attributed to reflux directly or due to the consequences of GERD pathogenesis and/or complications merits further investigations, and development of step-up or step-down therapies, rather than prolonging therapy duration. Thus phenotype-based management approaches offer promise as potential mechanistic targets can be addressed. Owing to variability in clinical presentation,

changing pathophysiology during maturation, and/or presence of co-morbidities (respiratory or neurologic), empiric medical or surgical therapies have limited role in clinical practice. As multiple NICUs across the USA clinically diagnose GERD without objective testing with prolonged therapies,^{4,6} our study findings suggest that evidence-based GERD diagnosis and follow-up maybe beneficial. This approach can be generalizable to non-verbal older patients. Clinical history from crib-side caregivers, parent perceptions of symptoms, and prescription of acid-suppressive drugs or feeding-modification strategies are insufficient to make a conclusive diagnosis of GERD or of eating difficulties. As an example, arching and irritability events are the most common presenting symptoms and occurred in 100% of infants even in those who showed no sensitivity (S_{None} group). Arching and irritability can occur due to the method of airway support ($p = 0.0013$) with infants on NCPAP displaying a greater number of arching/irritability events. Despite observing that $S_{\text{Acid+Bolus}}$ and S_{Bolus} groups showed a greater number of arching/irritability events/day compared to S_{None} ($p = 0.01$ and 0.03 , respectively), overall GERD phenotypes did not show a significant relationship with the number of arching/irritability symptoms/day ($p = 0.0645$). Thus arching and irritability does not appear to represent a GER-specific symptom; developmental neuropathologies are considerations for alternative differential mechanisms.

There are limitations with our study

(1) Results are reported from consecutive pH-impedance studies and heterogeneous patient pool can be expected. Despite using multivariate models to control for this heterogeneity, further prospective studies are required to confirm results and for the development of universally accepted GERD diagnostic criteria and therapeutic strategies in NICU infants. Such studies require a large sample of infants, and to speed up large-scale implications, multi-center trials are needed. (2) As common in adult studies, SAP values are subject to proper documentation that may significantly impact accuracy of results. We have attempted to mitigate this limitation by having a nurse or nurse assistant at the bedside for the study duration whose sole responsibility was to document symptoms. Further prospective studies are needed to test whether the SAP-positive symptoms are truly ameliorated with therapies. Presence of significant cardio-respiratory events might require concurrent cardio-respiratory monitoring in conjunction to pH-impedance study. (4) As this was not designed as treatment study, information on diet (breast milk or formula) and changes to diet were not available. (5) To generalize applications in the NICU setting, specific training with data acquisition and data analytical protocols is needed to reproduce results. However, the current study provides pathophysiological explanation for symptoms.

In summary, we noted that the prevalence of sensitivity to acid alone is rare. The esophageal acid exposure index did not differ significantly between the four phenotypes. Bolus-sensitive phenotypes (S_{Bolus} , $S_{\text{Acid+Bolus}}$) had more aerodigestive symptoms/day compared to infants with no sensitivity. Arching/irritability is common among all infants, and the multivariate model showed that there are factors apart from GERD phenotypes, BPD, neuropathology, methods of feeding, and breathing that may impact the frequency of arching/irritability events. Some infants with normal ARI exhibited sensitivity to GER, while some with no sensitivity to GER had abnormal ARI. Having symptoms due to acid GER only could indicate chemosensitivity. The bolus ascent or the composition of the bolus can activate esophageal mechano-distension or laryngeal chemoreflex and aerodigestive reflexes or symptoms that increase alertness by engaging vagal mechanisms. We have provided proof of this concept in our prior provocative pharyngo-esophageal motility studies.^{16,21} Hence, mechanosensitivity occurs when bolus GER causes symptoms. Symptoms due to acid+bolus GER could indicate a mechano- and chemosensitivities. We propose that phenotyping of GERD based on

esophageal sensitivity to acid+bolus on pH-impedance monitoring can help develop therapeutic strategies.

In conclusion, the basis for troublesome symptoms should be defined objectively in the context of persistent physiological derangements, so as to permit individualized and well-targeted therapies. Future directions to therapeutic targets may include modification of inflammation, acid suppression, modification of bolus migration, personalization of feeding and GERD management strategies, feeding regulation, and behavioral modification of patients and providers, aiming for clinically meaningful outcomes. Multi-center randomized controlled trials based on objective criteria and definitions of disease instrumentation based on results of tests are needed in order to categorize phenotypes first and, based on that, develop rational therapies for moderate or severe GERD and feeding/aerodigestive difficulties.

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AUTHOR CONTRIBUTIONS

S.R.J. conceptualized and designed the study, secured funding and IRB approval, performed studies, interpreted data, drafted the initial manuscript, and critically reviewed and revised the manuscript with approval of the final version as submitted. Z.A.S. performed studies, data analysis and interpretation, manuscript writing from initial till final submission, and approved the final manuscript as submitted. K.A.H.-K. performed studies, conceptualized and performed data analysis, interpreted data, manuscript writing from initial till final submission, and approved the final manuscript as submitted. V.P. performed data validation, statistical analysis and interpretation, critical review and manuscript writing, and approved the final manuscript as submitted. I.K.G. performed studies, data interpretation, critical review and manuscript writing, and approved the final manuscript as submitted. C.D.L. interpreted data, critical review of the manuscript for important intellectual content, and manuscript writing. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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REFERENCES

- Vandenplas, Y. et al. Pediatric gastroesophageal reflux clinical practice guidelines: joint recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN). *J. Pediatr. Gastroenterol. Nutr.* **49**, 498–547 (2009).
- Rosen, R. et al. Pediatric Gastroesophageal Reflux Clinical Practice Guidelines: Joint Recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. *J. Pediatr. Gastroenterol. Nutr.* **66**, 516–554 (2018).
- Davidson, G. et al. Efficacy and safety of once-daily esomeprazole for the treatment of gastroesophageal reflux disease in neonatal patients. *J. Pediatr.* **163**, 692.e82–698.e82 (2013).
- Jadcherla, S. R. et al. Practice variance, prevalence, and economic burden of premature infants diagnosed with GERD. *Hosp. Pediatr.* **3**, 335–341 (2013).
- Slaughter, J. L., Stenger, M. R., Reagan, P. B. & Jadcherla, S. R. Neonatal histamine-2 receptor antagonist and proton pump inhibitor treatment at United States Children's hospitals. *J. Pediatr.* **174**, 63.e3–70.e3 (2016).
- D'Agostino, J. A., Passarella, M., Martin, A. E. & Lorch, S. A. Use of gastroesophageal reflux medications in premature infants after NICU discharge. *Pediatrics* **138**, e20161977 (2016).

7. Omari, T. et al. Pharmacokinetics and acid-suppressive effects of esomeprazole in infants 1–24 months old with symptoms of gastroesophageal reflux disease. *J. Pediatr. Gastroenterol. Nutr.* **60**(Suppl 1), S2–S8 (2015).
8. Peter, C. S., Wiechers, C., Bohnhorst, B., Silny, J. & Poets, C. F. Influence of nasogastric tubes on gastroesophageal reflux in preterm infants: a multiple intraluminal impedance study. *J. Pediatr.* **141**, 277–279 (2002).
9. Jadcherla, S. R. et al. Impact of feeding strategies on the frequency and clearance of acid and nonacid gastroesophageal reflux events in dysphagic neonates. *J. Parenter. Enter. Nutr.* **36**, 449–455 (2012).
10. Davidson, E., Hinton, D., Ryan-Wenger, N. & Jadcherla, S. Quality improvement study of effectiveness of cue-based feeding in infants with bronchopulmonary dysplasia in the neonatal intensive care unit. *J. Obstet. Gynecol. Neonatal Nurs.* **42**, 629–640 (2013).
11. Fox, D. et al. National trends and outcomes of pediatric gastrostomy tube placement. *J. Pediatr. Gastroenterol. Nutr.* **59**, 582–588 (2014).
12. Stey, A. M. et al. Hospital variation in rates of concurrent fundoplication during gastrostomy enteral access procedures. *Surg. Endosc.* **32**, 2201–2211 (2018).
13. Patel, A., Sayuk, G. S., Kushnir, V. M., Chan, W. W. & Gyawali, C. P. GERD phenotypes from pH-impedance monitoring predict symptomatic outcomes on prospective evaluation. *Neurogastroenterol. Motil.* **28**, 513–521 (2016).
14. Sivalingam, M. et al. Effects of esophageal acidification on troublesome symptoms: an approach to characterize true acid GERD in dysphagic neonates. *Dysphagia* **32**, 509–519 (2017).
15. Hasenstab, K. A. & Jadcherla, S. R. Respiratory events in infants presenting with apparent life threatening events: is there an explanation from esophageal motility? *J. Pediatr.* **165**, 250–255 e251 (2014).
16. Collins, C. R., Hasenstab, K. A., Nawaz, S. & Jadcherla, S. R. Mechanisms of aerodigestive symptoms in infants with varying acid reflux index determined by esophageal manometry. *J. Pediatr.* **206**, 240–247 (2019).
17. Jadcherla, S. R. et al. Significance of gastroesophageal refluxate in relation to physical, chemical, and spatiotemporal characteristics in symptomatic intensive care unit neonates. *Pediatr. Res.* **70**, 192–198 (2011).
18. Jadcherla, S. R. et al. Spatiotemporal characteristics of acid refluxate and relationship to symptoms in premature and term infants with chronic lung disease. *Am. J. Gastroenterol.* **103**, 720–728 (2008).
19. Jadcherla, S. R., Hanandeh, N., Hasenstab, K. A. & Nawaz, S. Differentiation of esophageal pH-impedance characteristics classified by the mucosal integrity marker in human neonates. *Pediatr. Res.* **85**, 355–360 (2019).
20. Mousa, H. M. et al. Esophageal impedance monitoring for gastroesophageal reflux. *J. Pediatr. Gastroenterol. Nutr.* **52**, 129–139 (2011).
21. Gupta, A. & Jadcherla, S. R. The relationship between somatic growth and in vivo esophageal segmental and sphincteric growth in human neonates. *J. Pediatr. Gastroenterol. Nutr.* **43**, 35–41 (2006).
22. Lopez-Alonso, M. et al. Twenty-four-hour esophageal impedance-pH monitoring in healthy preterm neonates: rate and characteristics of acid, weakly acidic, and weakly alkaline gastroesophageal reflux. *Pediatrics* **118**, e299–e308 (2006).
23. Vandenplas, Y., Goyvaerts, H., Helven, R. & Sacre, L. Gastroesophageal reflux, as measured by 24-hour pH monitoring, in 509 healthy infants screened for risk of sudden-infant-death-syndrome. *Pediatrics* **88**, 834–840 (1991).
24. Vandenplas, Y., Helven, R., Goyvaerts, H. & Sacre, L. Reproducibility of continuous 24 h oesophageal pH monitoring in infants and children. *Gut* **31**, 374–377 (1990).
25. Sankaran, J., Qureshi, A. H., Woodley, F., Splaingard, M. & Jadcherla, S. R. Effect of severity of esophageal acidification on sleep vs wake periods in infants presenting with brief resolved unexplained events. *J. Pediatr.* **179**, 42–48 e41 (2016).
26. Jadcherla, S. R., Hasenstab, K. A., Shaker, R. & Castile, R. G. Mechanisms of cough provocation and cough resolution in neonates with bronchopulmonary dysplasia. *Pediatr. Res.* **78**, 462–469 (2015).
27. Zaichkin, J. G. Neonatal resuscitation: Neonatal Resuscitation Program 7th Edition Practice Integration. *Crit. Care Nurs. Clin. North Am.* **30**, 533–547 (2018).
28. Weusten, B. L., Roelofs, J. M., Akkermans, L. M., Van Berge-Henegouwen, G. P. & Smout, A. J. The symptom-association probability: an improved method for symptom analysis of 24-hour esophageal pH data. *Gastroenterology* **107**, 1741–1745 (1994).
29. Omari, T. I. et al. Optimisation of the reflux-symptom association statistics for use in infants being investigated by 24-hour pH impedance. *J. Pediatr. Gastroenterol. Nutr.* **52**, 408–413 (2011).
30. Richter, J. E. & Rubenstein, J. H. Presentation and epidemiology of gastroesophageal reflux disease. *Gastroenterology* **154**, 267–276 (2018).
31. Kahrilas, P. J. et al. Chronic cough due to gastroesophageal reflux in adults: CHEST Guideline and Expert Panel Report. *Chest* **150**, 1341–1360 (2016).
32. Orenstein, S. R. & Orenstein, D. M. Gastroesophageal reflux and respiratory disease in children. *J. Pediatr.* **112**, 847–858 (1988).
33. Lee, A. L. & Goldstein, R. S. Gastroesophageal reflux disease in COPD: links and risks. *Int. J. Chron. Obstruct. Pulmon. Dis.* **10**, 1935–1949 (2015).
34. Cohen Sabban, J. et al. Low-impedance baseline values predict severe esophagitis. *J. Pediatr. Gastroenterol. Nutr.* **65**, 278–280 (2017).
35. Sacco, O., Silvestri, M., Ghezzi, M., Capizzi, A. & Rossi, G. A. Airway inflammation and injury in children with prevalent weakly acidic gastroesophageal refluxes. *Respir. Med.* **143**, 42–47 (2018).
36. Babaei, A. et al. Impaired upper esophageal sphincter reflexes in patients with supraesophageal reflux disease. *Gastroenterology* **149**, 1381–1391 (2015).
37. Shaker, R. et al. Effect of lower esophageal sphincter tone and crural diaphragm contraction on distensibility of the gastroesophageal junction in humans. *Am. J. Physiol. Gastrointest. Liver Physiol.* **287**, G815–G821 (2004).
38. Gastal, O. L., Castell, J. A. & Castell, D. O. Frequency and site of gastroesophageal reflux in patients with chest symptoms. Studies using proximal and distal pH monitoring. *Chest* **106**, 1793–1796 (1994).
39. Jadcherla, S. R., Gupta, A., Stoner, E., Fernandez, S. & Shaker, R. Pharyngeal swallowing: defining pharyngeal and upper esophageal sphincter relationships in human neonates. *J. Pediatr.* **151**, 597–603 (2007).
40. Gupta, A. et al. Effect of postnatal maturation on the mechanisms of esophageal propulsion in preterm human neonates: primary and secondary peristalsis. *Am. J. Gastroenterol.* **104**, 411–419 (2009).
41. Pena, E. M. et al. Lower esophageal sphincter relaxation reflex kinetics: effects of peristaltic reflexes and maturation in human premature neonates. *Am. J. Physiol. Gastrointest. Liver Physiol.* **299**, G1386–G1395 (2010).
42. Jadcherla, S. R., Duong, H. Q., Hoffmann, R. G. & Shaker, R. Esophageal body and upper esophageal sphincter motor responses to esophageal provocation during maturation in preterm newborns. *J. Pediatr.* **143**, 31–38 (2003).
43. Jadcherla, S. R., Gupta, A., Coley, B. D., Fernandez, S. & Shaker, R. Esophago-glottal closure reflex in human infants: a novel reflex elicited with concurrent manometry and ultrasonography. *Am. J. Gastroenterol.* **102**, 2286–2293 (2007).
44. Jadcherla, S. R., Chan, C. Y., Fernandez, S. & Splaingard, M. Maturation of upstream and downstream esophageal reflexes in human premature neonates: the role of sleep and awake states. *Am. J. Physiol. Gastrointest. Liver Physiol.* **305**, G649–G658 (2013).
45. Jadcherla, S. R. et al. Upper and lower esophageal sphincter kinetics are modified during maturation: effect of pharyngeal stimulus in premature infants. *Pediatr. Res.* **77**, 99–106 (2015).
46. Peter, C. S., Sprodowski, N., Bohnhorst, B., Silny, J. & Poets, C. F. Gastroesophageal reflux and apnea of prematurity: no temporal relationship. *Pediatrics* **109**, 8–11 (2002).
47. Wenzl, T. G. et al. Association of apnea and nonacid gastroesophageal reflux in infants: Investigations with the intraluminal impedance technique. *Pediatr. Pulmonol.* **31**, 144–149 (2001).
48. Rossor, T., Andradi, G., Ali, K., Bhat, R. & Greenough, A. Gastro-oesophageal reflux and apnoea: is there a temporal relationship? *Neonatology* **113**, 206–211 (2018).
49. Hasenstab, K. A., Sitaram, S., Lang, I. M., Shaker, R. & Jadcherla, S. R. Maturation modulates pharyngeal-stimulus provoked pharyngeal and respiratory rhythms in human infants. *Dysphagia* **33**, 63–75 (2018).
50. Jadcherla, S. R. et al. Definition and implications of novel pharyngo-glottal reflex in human infants using concurrent manometry ultrasonography. *Am. J. Gastroenterol.* **104**, 2572–2582 (2009).
51. Jadcherla, S. R. Manometric evaluation of esophageal-protective reflexes in infants and children. *Am. J. Med.* **115**, 157s–160s (2003).
52. Jadcherla, S. R. & Shaker, R. Esophageal and upper esophageal sphincter motor function in babies. *Am. J. Med.* **111**, 64–68 (2001).
53. Djeddi, D., Cantin, D., Samson, N. & Praud, J. P. Nasal continuous positive airway pressure inhibits gastroesophageal reflux in newborn lambs. *PLoS ONE* **9**, e107736 (2014).
54. Jadcherla, S. R. et al. Effect of nasal noninvasive respiratory support methods on pharyngeal provocation-induced aerodigestive reflexes in infants. *Am. J. Physiol. Gastrointest. Liver Physiol.* **310**, G1006–G1014 (2016).
55. Eichenwald, E. C. & Committee on Fetus and Newborn. Diagnosis and Management of Gastroesophageal Reflux in Preterm Infants. *Pediatrics* **142**, e20181061 (2018).