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Differentiating Esophageal Sensitivity Phenotypes using pH-Impedance in Intensive Care Unit Infants referred for Gastroesophageal Reflux Symptoms

Sudarshan R. Jadcherla^{1,2}, Zakia Sultana¹, Kathryn A. Hasenstab-Kenney¹, Varsha Prabhakar¹, Ish K. Gulati¹, Carlo Di Lorenzo^{2,3}

¹The Neonatal and Infant Feeding Disorders Program, Center for Perinatal Research, Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH

²Pediatric Gastroenterology and Nutrition; Department of Pediatrics; The Ohio State University College of Medicine, Columbus, OH

³Pediatric Gastroenterology and Nutrition, Department of Pediatrics; Nationwide Children's Hospital, Columbus, OH

Abstract

OBJECTIVE: 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-hour pH-impedance. Symptom associated probability (SAP) for acid and bolus components defined esophageal sensitivity: 1) S_{Acid} as SAP 95% for acid (pH<4), 2) S_{Bolus} as SAP 95% for bolus, 3) $S_{\text{Acid+Bolus}}$ as SAP 95% for acid and bolus, or 4) S_{None} as SAP<95% for acid and bolus.

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CORRESPONDING AUTHOR: Sudarshan R. Jadcherla, MD, FRCPI, DCH, AGAF, Professor of Pediatrics and Associate Division Chief of Neonatology, Academics, Principal Investigator, Innovative Infant Feeding Disorders Research Program, Abigail Wexner Research Institute at Nationwide Children's Hospital, 575 Children's Crossroads, Columbus, OH 43215, **Phone:** 614-355-6643, Sudarshan.Jadcherla@nationwidechildrens.org.

AUTHOR CONTRIBUTIONS:

Dr. Sudarshan R. Jadcherla 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. Zakia A Sultana performed studies, data analysis and interpretation, manuscript writing from initial till final submission, and approved the final manuscript as submitted. Kathryn A. Hasenstab-Kenney performed studies, conceptualized and performed data analysis, interpreted data, manuscript writing from initial till final submission, and approved the final manuscript as submitted. Varsha Prabhakar performed data validation, statistical analysis and interpretation, critical review and manuscript writing, and approved the final manuscript as submitted. Dr. Ish K. Gulati performed studies, data interpretation, critical review and manuscript writing, and approved the final manuscript as submitted. Dr. Carlo Di Lorenzo interpreted data, and 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.

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There are no disclosures (financial, professional, or personal) to declare for all authors.

CONSENT: Informed consent was obtained from parents prior to pH-impedance study.

CATEGORY OF STUDY: Clinical Translational Research

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 ($p<0.001$), 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.

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 2–30% among US neonatal intensive care units (NICU), amounting to an additional burden of over \$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 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-hour 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, Ohio, USA) for the evaluation of GERD. Inclusion criteria included infants that: 1) had 24-hour pH-impedance testing between 2012–2015 with 18 hours of analyzable pH-impedance data, and 2) were enterally fed time of evaluation, and 3) were < 60 weeks post menstrual age (PMA) at 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-hour 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 equations (21), 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 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 sec 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 per day, the longest duration, pH only events, and acid clearance time (23, 24). Impedance characteristics (14, 17–19, 26) 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, sec, 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 hour shifts, continuously for 24 hours for each patient. As previously published (14, 16–18, 27), 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 sec, heart rate <80 bpm 10 sec, or oxygen saturation <80% 10 sec, respectively (28). Individual symptom prevalence was counted if a symptom occurred at least once during the 24 hour 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 ≥ 95% indicating that the observed associations did not occur by chance, thus were likely caused by GER (14, 29, 30). SAP values were calculated using 2-minute windows, reflux was considered symptomatic when a reflux event occurred within 2-minute 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 (Figure 1). To clarify 1) *Sensitivity to acid only* (S_{Acid}) was defined as pH SAP ≥ 95% and impedance SAP < 95%, 2) *Sensitivity to bolus only* (S_{Bolus}) was defined as having pH SAP < 95% and impedance SAP ≥ 95%, 3) *Sensitivity to acid and bolus* ($S_{\text{Acid+Bolus}}$) was defined as pH SAP ≥ 95% and impedance SAP ≥ 95%, and 4) *No sensitivity* (S_{None}) was defined as pH SAP < 95% and impedance SAP < 95%.

Statistical Analysis—Kruskal-Wallis and Fishers exact tests were used to compare demographic characteristics, outcomes, prevalence and distribution of symptoms between groups (29). Dunn's multiple comparison test was used to perform multiple pairwise comparisons. pH-impedance characteristics were compared using Welch's ANOVA 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, BPD and neuropathology with the number of arching/irritability, coughs, emesis and sneezes per day. Partial F tests was used to examine the

significance of each variable in the model. Bonferroni adjustments were applied for all multiple comparisons. Effects of bronchopulmonary dysplasia and neuropathology were also analyzed. *P* values < 0.05 were considered statistically significant. Data are presented as Median (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 hours of pH-impedance data. APGAR scores, median (IQR) were 4 (1 – 9) at 1 minute and 7 (1 – 10) at 5 minutes. 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 ± 64 vs 144 ± 68 symptoms per 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 bronchopulmonary dysplasia (N=155) vs no bronchopulmonary dysplasia (N=124) symptom occurrence was increased (148 ± 72 vs 126 ± 60 symptoms per 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 acid reflux index >7% was 38%, and reflux events >100 was 18%.

Esophageal Sensitivity Groups

Demographics and prevalence of esophageal sensitivity groups are shown (Table 1, Figure 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 (Figure 3). Emesis prevalence was significantly greater with S_{Bolus} and $S_{\text{Acid+Bolus}}$ ($P < 0.05$ vs S_{None} , Figure 3A). Frequency of the following symptoms were increased (vs S_{None}): cough for S_{Bolus} and $S_{\text{Acid+Bolus}}$, sneezing for S_{Bolus} , and emesis for S_{Bolus} and $S_{\text{Acid+Bolus}}$ (all $P < 0.05$, Figure 3B). ARI severity among esophageal sensitivity groups is shown (Figure 4).

Comparing proportions of esophageal sensitivity (S_{Acid} : S_{Bolus} : $S_{\text{Acid+bolus}}$: S_{None}) for: 1) ARI $< 7\%$ (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) bronchopulmonary dysplasia (N=155) vs no bronchopulmonary dysplasia (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 ($\beta \pm \text{SE}$, -0.13 ± 0.03 , $p < 0.001$) but greater for infants on NCPAP (N=13, 2.13 ± 0.85 , $p < 0.01$) and greater for S_{Bolus} (0.77 ± 0.36) and $S_{\text{Acid+Bolus}}$ (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 $S_{\text{Acid+Bolus}}$ (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 $S_{\text{Acid+Bolus}}$ (0.56 ± 0.16) infants ($p < 0.001$). *Number of sneezes per day* is decreased for infants on tracheostomy (-1.12 ± 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 1st 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 ($S_{\text{Acid+Bolus}}$), or none (S_{None}) were distinguished. Given that the demographic comparisons are similar across the 4 phenotypes, the salient findings are as follows: 1) *Esophageal sensitivities*: Prevalence of S_{Acid} is rare, with S_{Bolus} or $S_{\text{Acid+Bolus}}$ 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 (ACT) was similar across all phenotype groups. 4) *ARI characteristics* specific for # of acid reflux events and those >5 min, longest acid reflux events and distal esophageal exposure were all increased in those with $S_{\text{Acid+Bolus}}$, compared to S_{None} . Additionally, 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 lesser at higher PMAs, and greater for infants on NCPAP and for S_{Bolus} and $S_{\text{Acid+Bolus}}$. 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 $S_{\text{Acid+Bolus}}$ but lesser in infants with tracheostomy. Number of emesis events/day were greater for S_{Bolus} and $S_{\text{Acid+Bolus}}$, 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, 31, 32). 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 (33, 34). The chronicity and severity of these symptoms may depend on the integrity of esophageal mucosal barrier (19), presence of esophageal or airway inflammation (35, 36), pharyngo-esophageal motility (15), upper esophageal sphincter and lower esophageal sphincter function (37, 38), 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 (31, 39). 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 bronchopulmonary dysplasia 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 (40, 41), LES relaxation reflex (42), UES contractile reflex (43, 44), and may present with arching and irritability depending on UES involvement and arousals (45), b) supra-esophageal mediated i.e., pharyngeal swallowing (40, 46), c) extra-esophageal mediated i.e. airway reflexes, laryngeal chemoreflex, glottal closure reflexes, stridor and bronchospasm (44). 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 hr-acid reflux index (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 4 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 (TLESR) 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 (47, 48). 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 per 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, 47, 49, 50).

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, protective and clearance responses. These include esophago-glottal closure reflex (44), pharyngo-glottal

closure reflex (51), cough reflex (52), pharyngeal reflexive swallowing (53), laryngeal chemo reflex (54), esophago-deglutition reflex (55), secondary peristalsis (54), upper esophageal sphincter contractile reflex (56), and lower esophageal sphincter responses (57, 58). 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, 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 SSI) 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 (59). 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 (60), 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, 61) are not indicated. Patients treated with acid-suppressive medicines are frequently exposed to prolonged treatment (1, 61). 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. Due to variability in clinical presentation, changing pathophysiology during maturation, and/or presence of comorbidities (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 per 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 per 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 is to document symptoms. Further prospective studies are needed to test if 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 a 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 a 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 per 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 which 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 chemo sensitivities. 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, 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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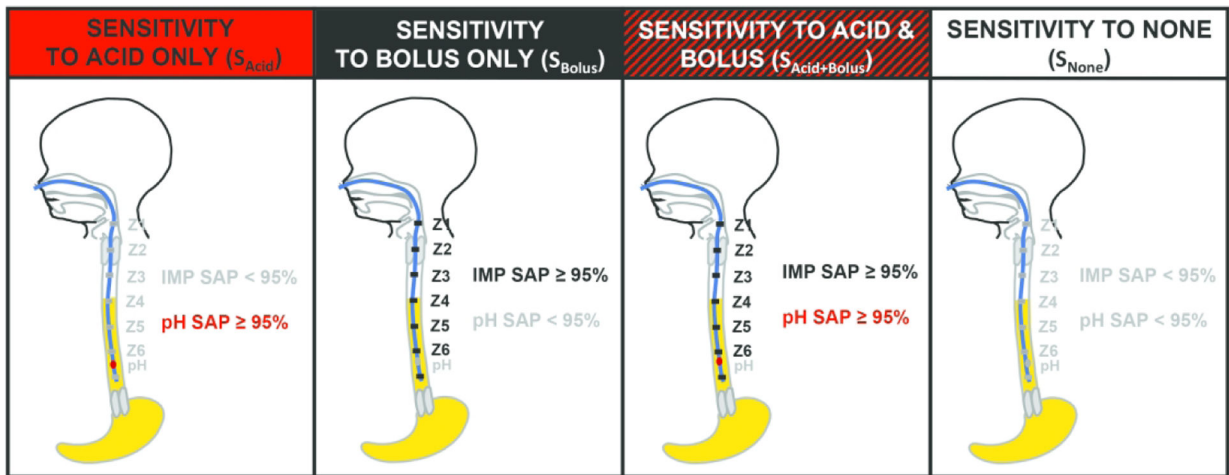
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Impact

- 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.



SAP ≥ 95% = Abnormal
 Note 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})

Figure 1. Classification of Esophageal Sensitivity Groups.

SAP ≥ 95% for acid events (as detected by pH) was classified as an acid sensitivity, and SAP ≥ 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).

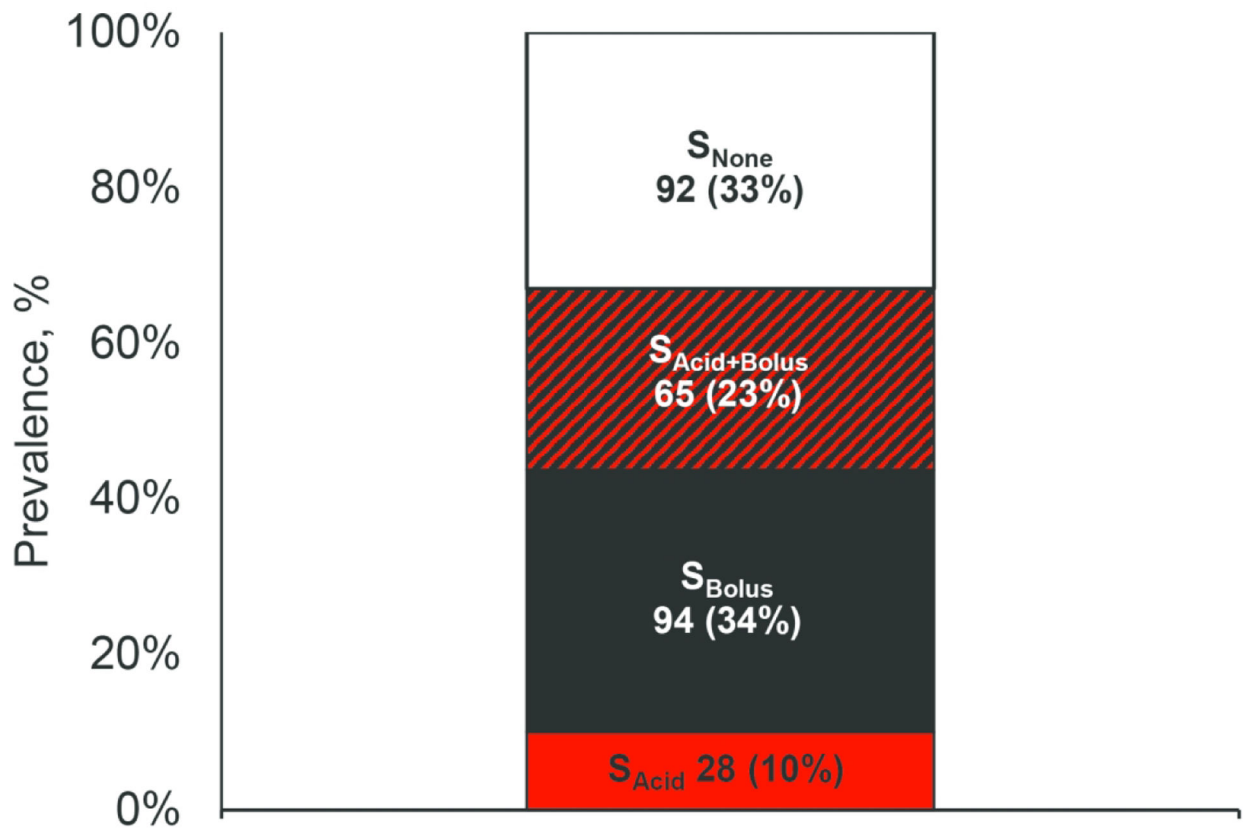


Figure 2. Prevalence of Esophageal Sensitivity Categories.

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%.

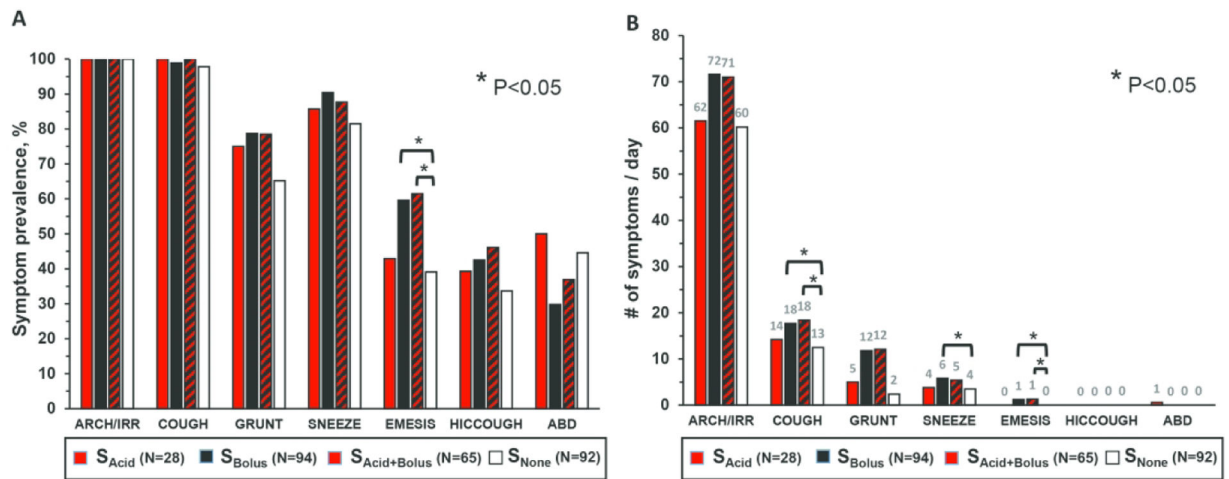


Figure 3. Symptom prevalence and type in relation to Esophageal Sensitivity categories.
A) Symptom prevalence (%). Nearly all infants exhibited arching/irritability (100%) and cough (98.9%). Emesis was increased in infants with any bolus sensitivity vs no sensitivity.
B) Symptom magnitude (# symptoms / 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.

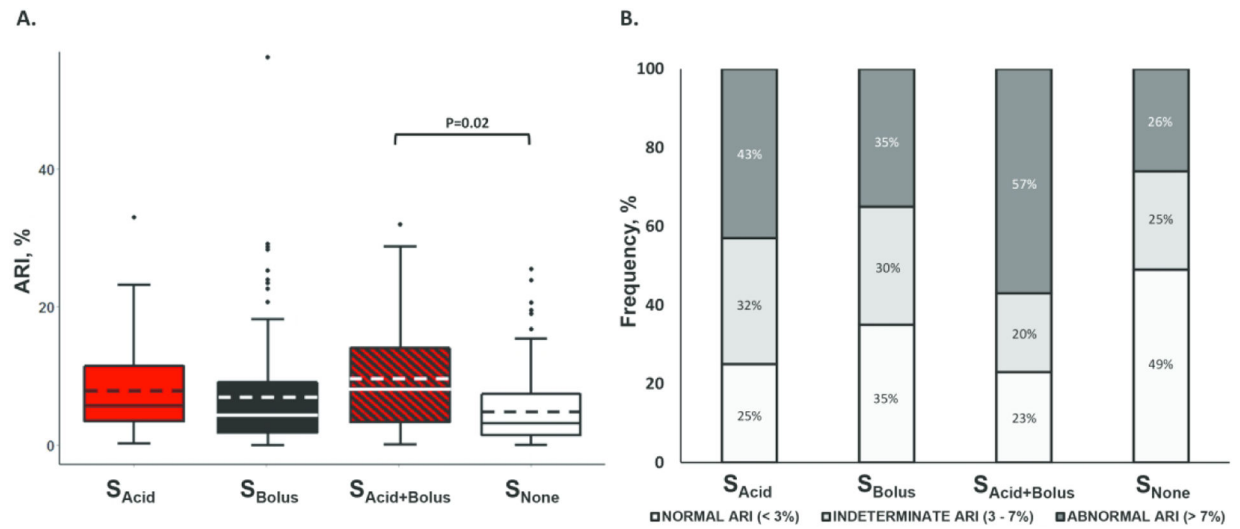


Figure 4. Distribution of acid reflux index (ARI) severity as a continuous variable (A) and as categorical variable (B) across the Esophageal Sensitivity categories.

A) ARI was increased in S_{Acid+Bolus} sensitivity. B) ARI distributions did not significantly differ between groups. SAP values > 95% were observed in even normal ARI groups. However clinically, 57% of those with S_{Acid+Bolus} have abnormal ARI.

Table 1.

Clinical and Outcome Characteristics Distributed by Esophageal Sensitivity Groups.

Characteristics	Overall	Sensitivity Group			P-value	
		S _{Acid} (N=28)	S _{Bohus} (N=94)	S _{Acid+Bohus} (N=65)		
At Birth						
Gestational age, wks	29 (26 – 34)	28 (25 – 31)	28 (26 – 33)	29 (26 – 33)	30 (26 – 34)	0.5
Premature Birth (< 37.0 wks), %	89	89	88	92	86	0.7
Gender, male, %	50	50	50	52	48	1
At Evaluation						
Postmenstrual age, wks	42 (40 – 45)	43 (40 – 45)	43 (41 – 46)	42 (41 – 45)	42 (40 – 45)	0.9
Chronologic age, wks	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 %.

* P<0.05 vs No sensitivity group. Note trach-Tracheostomy, NCPAP-Nasal Continuous Positive Airway Pressure, NC-Nasal Cannula.

Table 2. pH-impedance and Feeding Regimen Characteristics by Esophageal Sensitivity Group.

Characteristics	Overall	Sensitivity Group			P-value	
		S _{Acid} (N=28)	S _{bolus} (N=94)	S _{Acid+Bolus} (N=65)		
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, sec	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 \pm SD, % or Median (IQR) and BCT as Mean \pm SE.

* P<0.05 vs No sensitivity group.

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Table 3.

Significance of multivariate regression analysis variables on number of symptoms per day

Variables	Arch ing/Irritability per day	Coughs per day	Emesis per day	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

*p<0.05,

**p<0.001 on outcome. 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.

Table 4.

Summary of Multivariate regression analysis for predicting 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+Bohus}	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 _{Bohus}	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 SNone phenotype and did not have BPD or Neuropathology at evaluation was the reference. * and ** 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 SNone 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 SNone phenotype and did not have BPD or Neuropathology at evaluation.