

Characterization of upper gastrointestinal motility in infants with persistent distress and non-IgE-mediated cow's milk protein allergy

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ABSTRACT

Background: Persistent crying in infancy is common and may be associated with gastroesophageal reflux disease (GERD) and/or non-IgE mediated cow's milk protein allergy (CMPA). We aimed to document upper gastrointestinal motility events in infants with CMPA and compare these to findings in infants with functional GERD.

Methods: Infants aged 2-26 weeks with persistent crying, GERD symptoms and possible CMPA were included. Symptoms were recorded by 48-hour cry-fuss chart and validated reflux questionnaire (IGERDQ). Infants underwent a blinded milk elimination-challenge sequence to diagnose CMPA. GERD parameters and mucosal permeability were assessed by 24-hour pH-impedance monitoring before and after cow's milk protein (CMP) elimination. ¹³C-octanoate breath testing for gastric emptying dynamics, dual-sugar intestinal permeability, fecal calprotectin and serum vitamin D were also measured.

Results: Fifty infants (mean age 13 ± 7 weeks; 27 male) were enrolled. Based on CMP elimination-challenge outcomes, 14 (28%) were categorized as non-IgE-mediated CMPA, and 17 (34%) were not allergic to milk; 12 infants with equivocal findings and 7 with incomplete data were excluded. There were no baseline differences in GERD parameters between infants with and without CMPA. In the CMPA group, CMP elimination resulted in a significant reduction in reflux symptoms, esophageal acid exposure (reflux index), acid clearance time, and an increase in esophageal mucosal impedance.

Conclusions: In infants with persistent crying, upper gastrointestinal motility parameters did not reliably differentiate between non-IgE-mediated CMPA and functional GERD. In the group with non-IgE-mediated CMPA, elimination of CMP significantly improved GERD symptoms, esophageal peristaltic function and mucosal integrity.

Key words: cow's milk allergy; gastroesophageal reflux disease; pH-impedance monitoring; esophageal motility; mucosal permeability.

What is known about this subject?

- Infant distress has been attributed to gastroesophageal reflux disease (GERD) and/or cow's milk protein allergy (CMPA).
- Clinical phenotypes are poorly defined and diagnostic markers are lacking.
- Misdiagnosis leads to unnecessary dietary restrictions or PPI overtreatment.

What are the new findings and/or what is the impact on clinical practice?

- Elimination of cow's milk protein (CMP) reduced esophageal acid reflux and increased mucosal baseline impedance, in keeping with improved or restored mucosal integrity.
- Increased mucosal permeability was associated with insufficient vitamin D levels.
- Candidate diagnostic markers did not reliably predict CMPA, and CMP elimination and re-challenge therefore remain the definitive diagnostic procedure.

INTRODUCTION

Persistent crying in infancy is a major cause of parental concern, particularly when associated with sleeping or feeding problems (1). Apart from behavioral factors, persistent crying has been attributed to a range of clinical diagnoses, including gastroesophageal reflux disease (GERD), cow's milk protein allergy (CMPA) and lactose intolerance. (1-4). However, the clinical phenotypes are poorly defined and reliable diagnostic markers are lacking.

Gastroesophageal reflux (GER) is the retrograde flow of gastric contents into the esophagus. GER is physiological in infancy. In some infants, reflux episodes may be abnormally increased (GER disease; GERD) and cause reflux esophagitis, leading to distressed behavior which may be associated with feeding difficulties. The relationship between functional GERD and CMPA-associated GERD remains unclear (5, 6). However, the accurate differentiation between both conditions is important as a misdiagnosis may lead to either unnecessary dietary restrictions or overtreatment with acid-suppressing medications (7-10). The recent trend of prescribing proton pump inhibitors (PPI) in infancy with persistent crying is concerning as it has been linked to a range of potential adverse outcomes (11-16).

We performed a prospective study of infants with persistent crying, frequent regurgitation and/or feeding difficulties which assessed upper gastrointestinal motility findings in relation to a diagnosis of CMPA. Our primary hypothesis was that infants with CMPA could be differentiated from those with functional GERD based on pH-impedance monitoring finding. We also explored the role of adjunctive testing for gastric dysmotility, impaired small intestinal permeability, gastrointestinal inflammation and vitamin D status.

METHODS

The study was approved by the Human Research Ethics Committee of the Women's & Children's Health Network (No. 2018 conducted between 05/11 - 01/15).

The phases of the study protocol are summarized below (see Figure 1).

Phase I: Baseline assessment

Infants aged 2-26 weeks referred for specialist investigation of parent-reported persistent crying, vomiting and/or food refusal were enrolled. Informed consent was obtained. Infants underwent a detailed assessment at baseline, including physical examination, atopy patch testing to cow's milk, as well as measurement of milk-specific serum IgE and serum vitamin D levels. Any PPI medications were ceased at enrolment. Formula-fed infants were commenced on a cow's milk formula (S26™, Wyeth Australia Pty. Ltd.) under nurse supervision. Breast feeding mothers returned to an unrestricted diet, including fresh cow's milk and other milk products. Baseline testing was scheduled after 3-5 days to allow for a wash-out of PPI effect. Parents kept a 48-hr cry-fuss chart and completed a GERD questionnaire. A ¹³C-octanoate breath test gastric emptying was performed. A stool sample was taken to measure fecal calprotectin as a marker for intestinal inflammation, and an intestinal dual sugar test (lactulose-rhamnose ratio) assessed small intestinal permeability.

Phase II: Cow's milk protein elimination phase and re-assessment

Following baseline assessments, infants underwent a 10-14-day CMP elimination trial. Formula-fed infants commenced amino acid-based formula (AAF; EleCare®, Abbott Nutrition, Australia). Mothers of fully breastfed infants strictly eliminated cow's milk, soy and egg from their diet and took an oral calcium supplement (1.2g/day). Mothers received

dietician education and completed a food diary to monitor adherence. Assessments were repeated at the end of the CMP elimination phase.

Phase III: Diagnostic CMP challenge

Within seven days of completing Phase II, infants underwent two food challenges (inclusion/exclusion of CMP) to assess for CMPA, separated by a one-week wash-out period (17). Cry-fuss charts were documented for 48 hours at the end of each challenge period (Days 6 and 7 of the CMP-free and CMP-containing phases).

Formula-fed infants: Infants were randomly allocated to receive a CMP-free or CMP-containing formula for a target period of 7 days (minimum 3 days), in cross-over fashion. The first feed of each challenge was given under nurse supervision, and parents were blind to treatment allocation. Blinding was achieved by disguising the CMP-containing formula in AAF (one-part cow's milk-based formula: two parts of AAF). Pre-mixed formula powder (CMP-containing AAF and pure AAF) was pre-prepared in coded tins (Pharmaceutical Packaging Professionals Pty. Ltd., Australia).

Exclusively breast-fed infants: Infants underwent challenges in randomized order, but mothers could not be blinded to diet allocation. For the CMP exclusion, mothers underwent strict dietary exclusion of CMP, soy and egg (as above). For the CMP challenge, mothers were asked to include 3 serves of cow's milk products per day in their diet (1 serve defined as 1 cup fresh cow's milk, 200g yogurt or custard and/or 2 slices of cheese).

Diagnostic assessment of milk challenge outcome: The 48-hr cry-fuss times were utilized for determining the effects of dietary CMP elimination and challenge. A diagnosis of CMPA required: (1) a $\geq 33\%$ decrease in cry-fuss time from baseline to the end of the initial treatment phase i.e. CMP exclusion (Phase II), followed by (2) a $\geq 33\%$ rebound in cry-fuss time after CMP challenge (Phase III), while not displaying a clinical rebound during the

control challenge with AAF. Infants with lesser cry-fuss changes were categorized as 'equivocal'. Infants who showed no improvement during the initial elimination phase or who had discordant challenge results were classified as 'non-responders' and deemed to not suffer from CMPA.

Study Assessments

Cry-fuss charts

Parental cry-fuss chart (18) was completed for a minimum of 48h and scored by the project dietician or nurse for the average 24h cry-fuss time. The upper 95% confidence limit of normal for this instrument is 130 min/day (19).

Allergy testing

Cow's milk-specific serum IgE was measured by ImmunoCAP™ assay (ThermoFisher Scientific Australia). Atopy patch testing (APT) was performed at baseline using a 12mm Finn chamber (20). One drop of fresh, full-cream cow's milk was soaked onto filter paper and fixed to the upper right quadrant of the back under an occlusive dressing. A control patch with sterile 0.9% saline was also applied. Patches were left in place for 48 hours and then removed. At 72 hours, the exposed skin area was examined for evidence of erythema, papules and induration, compared to the control patch (photograph). A positive test was defined by induration beyond the Finn chamber margin and presence of at least four papules (20).

GERD Symptoms

Parents completed a validated infant GERD questionnaire (IGERDQ). A score $\geq 16/42$ was considered abnormal (21).

Assessment of GERD and esophageal mucosal impedance

Gastroesophageal reflux severity was determined by 24h pH-multi-channel intraluminal-impedance probe (pH-MII; *ComforTec* single use infant catheter, *Sleuth* data logger and *Bioview* automated analysis software; Sandhill Scientific, Highlands Ranch, CO, USA). The pH sensor was placed at the level of the third vertebra above the diaphragm, confirmed by a chest X-ray. Acid exposure in the distal esophagus was defined by the reflux index (% time with pH <4). Abnormal esophageal acid exposure was defined as a reflux index $\geq 11.7\%$ (22). Impedance-detected bolus reflux events were manually verified. Abnormal liquid GER frequency was defined as $>100/24$ hours (23-25).

The mucosal impedance baseline was calculated at each of the six impedance segments (Z1 proximal to Z6 distal), using purpose-designed software created using Matlab (MathWorks, Natick, MA). The software excludes sudden drops and rises in impedance values and calculates the median impedance baseline for the entire study period. The method has been validated against manual analysis (26, 27).

Gastric emptying testing

Half gastric emptying time ($GET_{1/2}$) was measured by ^{13}C -octanoate breath test. ^{13}C labelled Na-octanoate (50mg) was added to the feed. Breath samples were collected and analyzed for $^{13}\text{CO}_2$ content by isotope ratio mass spectrometry. The $^{13}\text{CO}_2$ excretion rate was used to calculate $GET_{1/2}$ using the established regression model (28). A $GET_{1/2} \geq 71$ min was considered as abnormally delayed (based on internal laboratory standards).

Dual-sugar intestinal permeability testing

Intestinal permeability (ISP) was measured using the lactulose-L-rhamnose dual sugar test. A single blood sample (1mL) was collected 90 min after the administration of lactulose (7.5mL) and rhamnose (1.1g) and analyzed for lactulose/rhamnose concentration by HPLC. A ratio of ≥ 7.8 was considered abnormal (29).

Fecal calprotectin testing

A stool sample (from diaper) was collected and stored at -80°C . Fecal calprotectin was determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Quantum Blue® Calprotectin rapid test, Bühlmann Laboratories AG, Switzerland). A fecal calprotectin of $\geq 621 \mu\text{g/g}$, corresponding with the upper 95% CI limit in infants 0-3months (30), was considered as evidence of intestinal inflammation.

Serum vitamin D

Serum vitamin D₂ (25-hydroxycholecalciferol) levels were measured by liquid chromatography tandem mass spectrometry. Insufficiency was defined as a serum vitamin D₂ level $< 60 \text{ nmol/L}$ (normal range 60-160 nmol/L). Infants found to be vitamin D insufficient were prescribed vitamin D oral supplements at 25 micrograms per day per standard clinical practice (OsteVitD oral liquid drops, Key Pharmaceuticals, Australia).

Statistical Analysis

Data are presented as mean $\pm$ standard deviation or median [inter-quartile range]. Statistical analysis was performed using SPSS Statistics 25 (IBM Corporation, USA). Subgroup comparisons for study outcomes were performed using independent samples Mann-Whitney U test and related samples Wilcoxon Signed Rank Test. Two-way contingency data were analyzed by Fisher's Exact test. Comparisons of mucosal impedance measures amongst

electrode segments and for time-point effects were performed using repeated measures ANOVA (General Linear Model). A p-value <0.05 represented statistical significance. Partial Eta Squared (η^2) indicated effect size.

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RESULTS

Baseline characteristics of cohort

Fifty infants (27 male) between 2-26 weeks of age (mean 13 ± 7) were enrolled. Baseline patient cohort characteristics are shown in Table 1. Thirty-seven of the infants (74%) were exclusively breastfed, 29 (58%) had received PPI therapy prior to enrolment, and 34% were vitamin D insufficient. At enrolment, all infants had abnormal cry-fuss times (mean 496 ± 248 min) and most (42, 84%) had abnormal GERD symptom scores (IGERDQ) (mean score 21 ± 6 ; abnormal ≥ 16)).

The final grouping of patients, based on the cow's milk protein elimination and challenge sequence (Figure 1) comprised fourteen infants (28%) who met the study criteria for CMPA, and 17 (34%) who had no response to CMP elimination or challenge. Twelve infants (24%) exhibited equivocal challenge outcomes. Seven infants (14%) had incomplete cry-fuss chart records and were excluded from further analysis.

Primary comparisons exploring differences in relation to CMPA were limited to the 31 infants (20, 65% male; age 13 ± 6 weeks) with conclusive challenge outcomes. Twenty-three (74%) of these infants were exclusively breastfed. All infants diagnosed with CMPA had abnormal cry-fuss duration (mean 506 ± 242 min/24 hrs), and 28 (90%) had abnormal IGERDQ scores (mean score 22 ± 5). No difference in Vitamin D status was seen between infants with or without CMPA (Table 1).

Atopy patch testing and serum specific CMP IgE testing

None of the infants with a conclusive CMPA challenge outcome had a history of immediate-type clinical reactions to CMP; these were classified as non-IgE-mediated CMPA. On serum specific CMP IgE testing, none of the infants had evidence of significant IgE sensitization

(sIgE to milk < 0.35 kU_A/L). Only one infant had a positive APT; this infant had also been identified as CMPA based on challenge outcome. The APT showed poor diagnostic accuracy for CMPA in this cohort due to an extremely low sensitivity (sensitivity 0.07, specificity 1.00, Fisher's Exact Test; p=0.452).

Gastroesophageal reflux assessment

At baseline, while exposed to CMP through breast milk or formula, there were no significant differences in GERD parameters between infants with and without CMPA (Table 1). Dietary exclusion of CMP was associated with a significant reduction in IGERDQ reflux symptom scores in both groups (Table 2), with a statistically greater reduction seen in the CMPA group.

Further interrogation of IGERDQ sub-components revealed that in the CMPA group, the reduction in scores following dietary CMP exclusion was driven by improvements in feeding-associated symptoms/behaviors (Supplemental Table 1, Supplemental Digital Content, <http://links.lww.com/MPG/B761>). Effects on individual infant behavior sub-component scores were also observed in the group without CMPA, a significant response to Q5. (*Does the baby have trouble gaining weight?*) was observed for both groups (p=0.050 for CMPA and p=0.019 for not CMPA). Scores for Q1. (*How often does the baby usually spit up?*) were consistently higher in infants with CMPA at both study time points (ON CMP 3 [3, 3] vs. 2 [1, 3], p=0.002 and OFF CMP 3 [2, 3] vs. 1 [1, 2], p=0.006 for CMPA and Not CMPA respectively).

In the CMPA group, 24hr pH-monitoring results showed that CMP exclusion resulted in a significant reduction the esophageal acid exposure (reflux index) and mean acid clearance time. However, there were no significant effects on the frequency of acid, non-acid and total

MII-detected GER episodes (Table 2 and Supplemental Table 1, Supplemental Digital Content, <http://links.lww.com/MPG/B761>).

Mucosal Impedance

At baseline, there were no significant differences in mucosal impedance between infants with and without CMPA (grand mean impedance segments Z1-Z6 was 2.2 (0.5) k Ω in non-CMPA vs. 2.1 (0.5) in CMPA group). Previous exposure to PPI did not influence mucosal impedance reading (grand mean impedance segments Z1-Z6 was 2.2 (0.5) k Ω in PPI-exposed infants vs. 2.1 (0.5) in those who had never been exposed). However, in infants with CMPA, the mucosal impedance increased following CMP exclusion (Figure 2). This effect was most prominent within the distal esophageal body. The difference in mean impedance was most striking across segments Z3-Z6 with an increase from 2.0 ± 0.5 k Ω while exposed to CMP to 2.2 ± 0.6 k Ω while avoiding CMPA (RM ANOVA $F=6.512$, $p=0.027$, medium η^2 effect size = 0.372).

The effects baseline of vitamin D status on mucosal impedance were examined by ANOVA for all electrode segments and across both time-points as repeated measures. Infants meeting the study definition of vitamin D insufficiency demonstrated significantly lower mucosal impedance values, irrespective of their CMPA status or exposure to CMP (impedance 1.9 ± 0.1 k Ω in vitamin D insufficient infants vs. 2.3 ± 0.1 k Ω in sufficient infants; $F=5.089$, $p=0.033$, η^2 effect size = 0.164).

Adjunctive testing for gastric dysmotility, impaired small intestinal permeability and gastrointestinal inflammation

The overall distributions of data on gastric emptying time, intestinal sugar permeability or faecal calprotectin are shown in Tables 1 and 2. The only difference seen between study groups was a significantly higher small intestinal permeability in the infants that did not suffer from CMPA. Gut permeability exceeded normal limits in 31% of non-CMPA infants. However, we were unable to define an underlying reason for the observed permeability increase (Table 1).

DISCUSSION

We examined the upper gastrointestinal motility characteristics of breast- and formula-fed infants under 26 weeks of age with persistent crying, frequent regurgitation and/or food refusal, in relation to a diagnosis of CMPA-induced or functional GERD. The current study applied a rigorous diagnostic protocol, involving a 2-week period of CMP elimination, followed by two single-blinded CMP challenges, in randomized order. This approach was in line with clinical guidelines for CMPA (17) and GERD in infants and children (31). None of the infants in our study categorized as CMPA had a history of immediate allergic reactions or significant IgE sensitization, and these infants were therefore categorized as suffering from ‘non-IgE-mediated CMPA’. As expected, APT and serum specific CMP IgE testing were not useful in making a diagnosis of non-IgE-mediated CMPA. In the CMPA group, a reduction in esophageal acid exposure and acid clearance time after elimination of CMP were observed, suggesting improved peristaltic function. However, objective measures of gastroesophageal reflux, gastric emptying dynamics, small intestinal permeability measures and fecal calprotectin at baseline did not reliably differentiate non-IgE-mediated CMPA from functional GERD.

In symptomatic infants, the reflux burden can be investigated by esophageal pH-MII monitoring. Our hypothesis was that a *negative* GERD diagnosis, based on esophageal pH-MII monitoring findings, would suggest CMPA over primary GERD as an underlying diagnosis. Whilst almost all infants with CMPA had normal esophageal acid exposure levels, many infants in the non-CMPA group were also normal. The low yield of positive pH-MII probe findings is perhaps not surprising given past reports that 2/3 of distressed infants with endoscopically proven esophagitis have normal esophageal acid exposure (32). Interestingly, the dietary elimination of CMP significantly reduced esophageal acid exposure in the CMPA

infants. This effect appeared to be mainly driven by improvements in esophageal acid clearance, suggesting improved esophageal peristaltic function.

Impedance is a direct measure of mucosal integrity. Low mucosal impedance is associated with dilated intercellular spaces, indicating increased transmucosal flux. Impedance is reduced in esophageal conditions with mucosal inflammation, including reflux esophagitis and eosinophilic esophagitis (33). In our study, dietary exclusion of CMP increased mucosal impedance in the CMPA group, in keeping with restoring mucosal integrity after CMP elimination. We speculate that the improvements in peristaltic function and mucosal integrity may be due to the resolution of an active allergic inflammatory process in the esophagus. These physiological findings may help explain the clinical improvement observed in the CMPA group after treatment with AAF. However, endoscopic biopsies were not available to assess the mucosal morphology and histology.

Vitamin D insufficiency has been proposed as a risk factor for food allergy, including CMPA (34). In our study, we did not observe an increased prevalence of vitamin D insufficiency in the infants with confirmed CMPA. Vitamin D levels correlated closely with mucosal impedance. Vitamin D is thought to play a role in maintaining intestinal barrier function by preserving tight junction proteins. In our study, vitamin D-insufficient infants demonstrated the lowest levels of impedance overall, and loss of esophageal mucosal barrier function appeared to be related to insufficient vitamin D levels.

Our study included other functional gut measures, including half gastric emptying time, fecal calprotectin and intestinal dual sugar permeability (lactulose-ramose ratio). Overall, these measures were not useful in differentiating CMPA from functional GERD. The only significant difference observed between infants with and without CMPA was a *lower* lactulose-ramose ratio suggesting, better intestinal barrier function in the CMPA group. The

clinical significance of this finding and underlying mechanism remains unclear, however, the abnormal gut permeability seen in 30% non-CMPA infants may suggest increased lower gut permeability due to other reasons, such as intestinal microbial dysbiosis.

Our study had several limitations. The study design included a broad range of gastrointestinal assessments and symptom surveys, and complete data sets were not available for all infants. The exclusion of 7 patients due to incomplete cry-fuss chart data and a higher than anticipated incidence of equivocal findings reduced the statistical power of this study and may have introduced bias. Breast-feeding mothers could not be blinded; however, their inclusion was essential to enable enough infants to be recruited. The small number of formula-fed infants in our study did not allow stratification by feeding mode due to sample size limitations. The effect of CMP exclusion on GERD parameters in formula-fed infants could therefore not be specifically examined. Finally, our study would have been strengthened had endoscopic biopsies been available to assess mucosal changes. The esophageal mucosal changes in infants with and without CMPA should be explored in further clinical studies.

In conclusion, in a cohort of infants under 6 months of age presenting with persistent distress and regurgitation/vomiting, approximately 30% were diagnosed with challenge-proven, non-IgE-mediated CMPA. None of the candidate diagnostic markers in this study tested reliably predicted CMPA, and CMP elimination and re-challenge therefore remain the definitive diagnostic procedure (17). Infants with CMPA demonstrated improvements in esophageal peristaltic function, as well as mucosal impedance. We speculate that these improvements are related to the resolution of esophageal allergic inflammation. The exact mechanisms involved in CMPA-associated persistent distress and GERD require further characterization.

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FIGURE LEGENDS

Figure 1. A. Study Design. The cry-chart response defining non-IgE CMPA was a $\geq 33\%$ improvement with cow's milk protein exclusion and a $\geq 33\%$ relapse and improvement on CMP/placebo. Infants with partial improvement were considered equivocal for CMA. Other patterns were considered not CMA. Infants with missing time points were excluded. **B.** Mean cry-fuss time for enrolled patients over the trial phases and grouped by CMP elimination-challenge outcomes; 14 responders (28%) were categorized as non-IgE-mediated CMPA, 17 non-responders (34%) were not allergic to milk, 12 infants had equivocal findings and 7 with incomplete data were excluded.

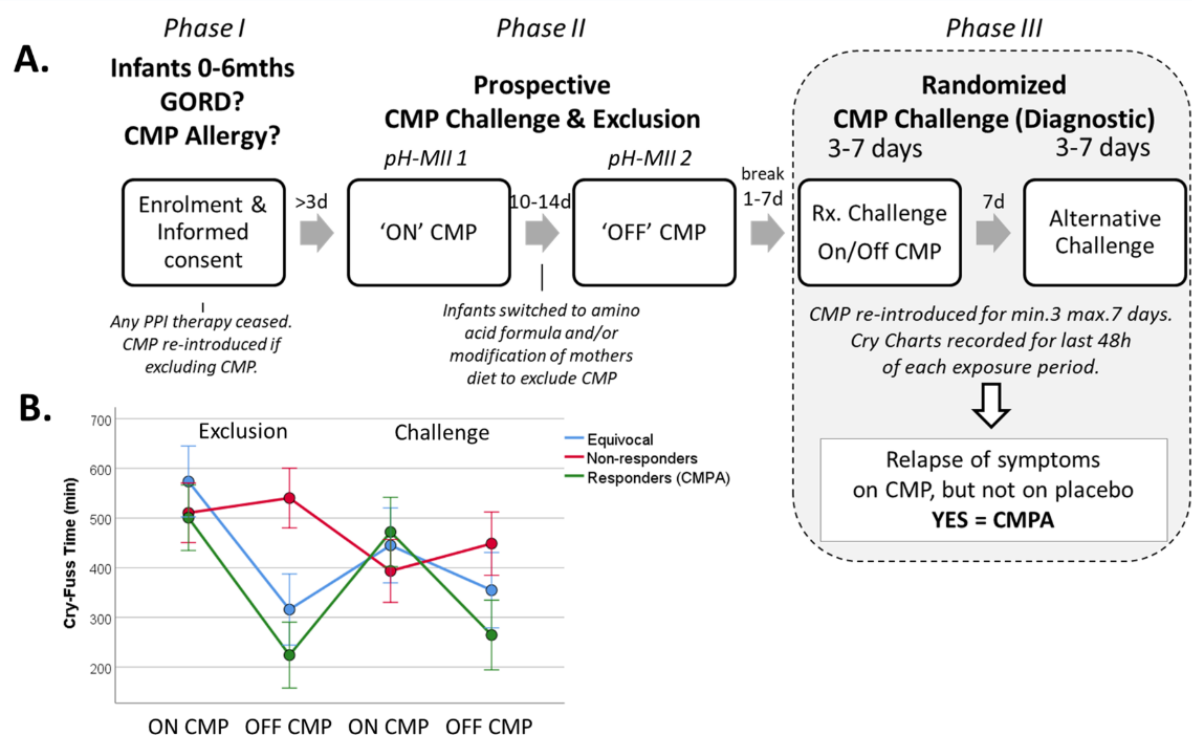


Figure 2. Effect of cow's milk protein exclusion on 24h mucosal baseline impedance measures along the esophageal body. Data are median [interquartile range]. * $p < 0.05$ for OFF vs. ON CMP using related samples Wilcoxon Signed Rank Test.

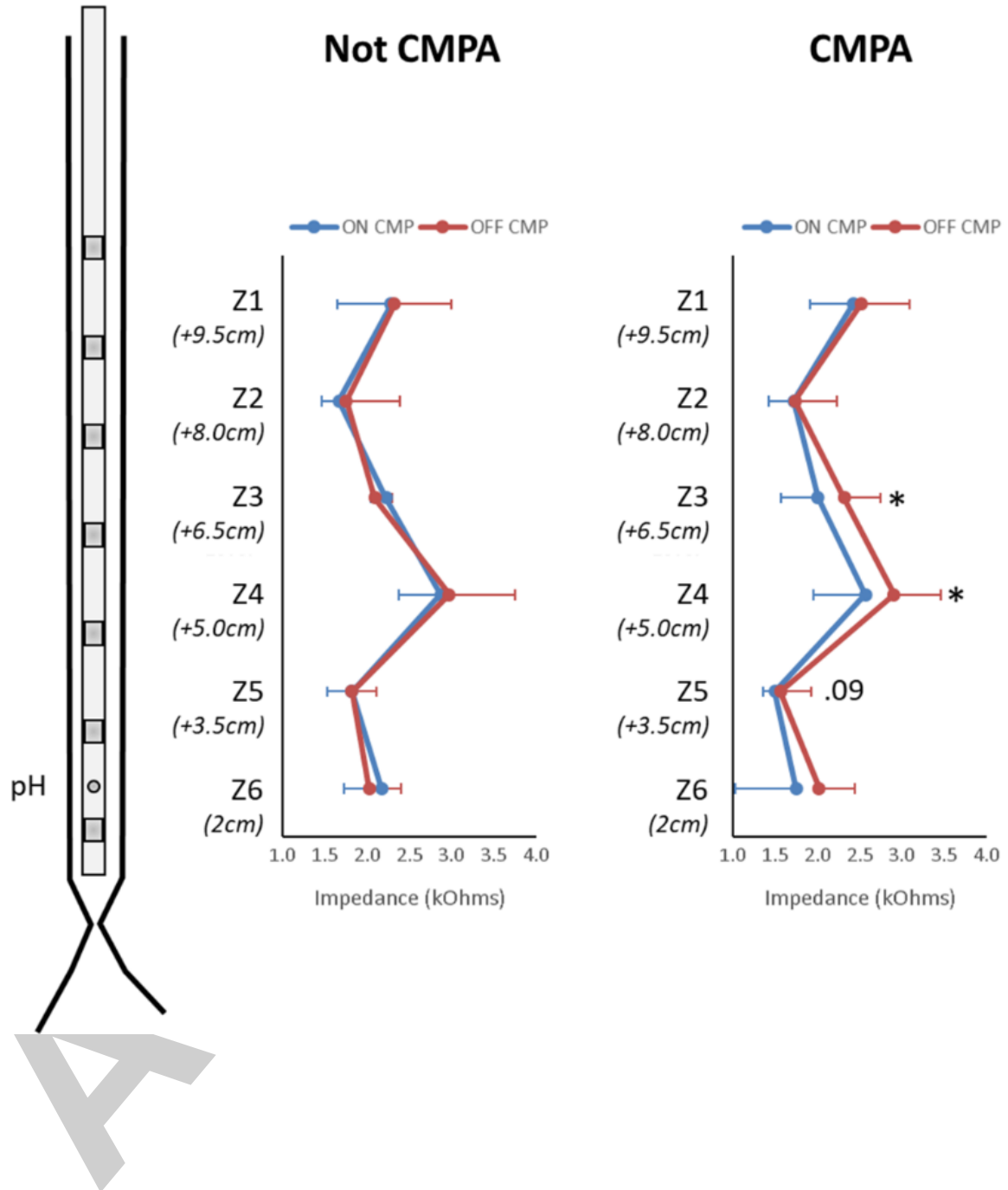


Table 1. Clinical baseline characteristics of all infants referred and the 31 infants who could be reliably diagnosed with and without CMPA. Data are mean $\pm$ SD (min, max) and median [interquartile range]. P-values for independent samples Mann-Whitney U test shown. Abbreviation: %ABN, %abnormal.

Baseline Characteristics	ALL INFANTS	
Demographics		
N, Gender (m:f)	50, 27:23	
Age (weeks)	13 ± 7 (2, 26)	
Height (cm)	59 ± 6 (49, 70)	
Weight (kg)	5.4 ± 1.4 (2.9, 8.5)	
WHO Z score (weight for age)	-1.2 ± 1.5 (-5.5, 1.8)	
Study Measures		% Abnormal
Cry-fuss time (min/day)	496 ± 248 (130, 1060)	100%
IGERDQ score (max. 42)	21 ± 6 (7, 32)	84%
Acid Exposure time pH<4 (%)	5.3 ± 4.9 (0.1, 21.9)	13%
	64 ± 22 (9, 125)	2%
Total MII GER Episodes (n)		
GE t1/2 (min)	72 ± 51 (20, 283)	30%
Vitamin D (nmol/l)	70 ± 37 (12, 201)	34%
Lactulose-rhamnose ratio	6 ± 4 (1, 22)	12%
Fecal Calprotectin (ug/g)	525 ± 660 (31, 4229)	24%

Baseline Characteristics	NOT CPMA		CPMA	p-value
Demographics				
N, Gender (m:f)	17, 9:8		14, 3:11	-
Age (weeks)	12 [6, 19]		12 [8, 17]	.493
Head circ. (cm)	56 [50, 64]		57 [53, 60]	.953
Weight (kg)	5.0 [3.8, 6.7]		5.5 [4.6, 5.8]	.681
WHO Z score	-0.9 [-1.4, -0.2]		-0.5 [-1.3, -0.1]	.468
Study Measures		% ABN	% ABN	
Cry-fuss time (min/day)	518 [405, 785]	100%	605 [300, 712]	.891
IGERDQ score (max. 42)		94%	24 [22, 25]	.297
Acid Exposure time pH<4 (%)	23 [21, 26]	19%	4.9 [2.7, 16.1]	.268
	2.6 [1.8, 7.0]	-	125 [91, 209]	.423
Mean Acid Clearance Time (s)	124 [82, 155]	12%	63 [50, 75]	.650
Total MII GER Episodes (n)	58 [42, 82]			
GE t1/2 (min)	57 [49, 81]	25%	71 [50, 143]	.351
Vitamin D (nmol/l)	65 [41, 86]	41%	75 [42, 93]	.710
Lactulose-rhamnose ratio	6 [5, 13]	31%	4 [4, 5]	.035
Fecal Calprotectin (ug/g)	397 [160, 832]	25%	263 [165, 464]	.320

Table 2. Effect of cow's milk protein exclusion on infants with and without CMPA. Data are median [interquartile range]. P-values for related samples Wilcoxon Signed Rank Test shown.

Effect of CMP Exclusion		Not CMPA		CMPA	
Study Measures	Difference	p	Difference	p	
Cry-fuss time (min/day)	30 [-195, 325]	.586	-360 [-543, -230]	-	
IGERDQ score (max. 42)	-5 [-10, 0]	.038	-8 [-12, -1]	.003	
Acid Exposure time pH<4 (%)	-1.0 [-2.9, 1.9]	.501	-2.0 [-3.6, 0.4]	.041	
Mean Acid Clearance Time (s)	2 [-45, 22]	.796	-14 [-129, 15]	.050	
Total MII GER Episodes (n)	-1 [-7, 8]	.878	0 [-3, 6]	.982	
GE t1/2 (min)	-5 [-16, 25]	.569	-45 [-107, 10]	.075	
Lactulose-rhamnose ratio	-1 [-3, 1]	.345	2 [-2, 3]	.062	
Fecal Calprotectin (ug/g)	-101 [-277, 414]	.653	62 [-109, 330]	.530	