

Effect of an Amino Acid-based Milk—Neocate[®]—on Gastro-Oesophageal Reflux in Infants Assessed by Combined Intraluminal Impedance/pH

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ABSTRACT

Cow's milk protein allergy-related gastro-oesophageal reflux (GOR) may be due to episodes of inappropriate lower oesophageal sphincter relaxation. The hypothetical physiological mechanism invoked to explain this involves a neurological consequence of the inflammatory infiltration induced from lamina propria vessels into the epithelial compartment. However, the efficacy of this approach has so far been examined with a physiologically-appropriate denominator to define the degree of GOR.

We intend to conduct an investigation of the influence of Neocate[®] an amino acid-based milk, on GOR in infants by using a combined pH and intraluminal oesophageal impedance measurement.

11 infants (6 male, median age 151 days, range 75 to 345 days) bottle-fed with either expressed breast milk or formula milk, with symptoms clinically suggestive of GOR, underwent two 24-hour studies of intra-oesophageal 6 channel impedance and dual-channel pH monitoring before and 14 days after establishment on Neocate[®] amino acid formula. Impedance/pH reflux data were recorded and analysed blind by one observer.

Ten out of 11 infant's parents reported a decrease in the reflux score ($p = 0.001$). The median number of: reflux events/hour (1.58 vs. 1.68); acid reflux events/hour (0.26 vs. 0.43); minimum distal or proximal pH; total acid clearance time per hour (time with pH below pH 4); reflux height proximally up the oesophagus; and total reflux duration per hour were not statistically significantly different with presenting formula compared to that after 14 days ingestion of Neocate[®].

This trial reveals a symptomatic improvement on while Neocate[®], but this improvement cannot be attributed to any positive effect on GOR as has been previously postulated. (Pediatr Asthma Allergy Immunol 2006; 19[4]:205–213.)

INTRODUCTION

GASTRO-OESOPHAGEAL REFLUX (GOR), the regurgitation of gastric contents into the oesophagus, is a physiological occurrence in all individuals. It is particularly frequent in infants and is probably largely

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physiological, usually resolving spontaneously.¹ GOR may become pathological when it causes symptoms such as heartburn, oesophagitis, acute life-threatening events, and respiratory disease.¹⁻³ Known consequences of GOR in infants are irritability, faltering growth, anaemia, and respiratory events such as aspiration pneumonia.^{1,2} Carre⁴ suggested that only 5 to 10% of children with vomiting or regurgitation in infancy continued to have symptoms after 4 years of age. More recent studies showed that 19% of infants with pathological GOR still have symptoms at 18 months of age.⁵ Currently available techniques for the study of non-acid (pH >4, <7) or alkaline reflux include ultrasound, bronco-alveolar lavage, scintigraphy, fluoroscopy, bilirubin monitoring, and pH monitoring.⁶⁻¹⁰ These methods fall far short of the ideal as they only measure a short time window, do not allow for symptom/event temporal correlation, or measure the wrong physiological denominator such as acidity only in the case of pH monitoring.

Oesophageal pH monitoring has gained general acceptance as the method for assessment of GOR in children and has been traditionally regarded as the first choice of investigation in infant GOR disease, especially if respiratory in manifestation.^{1,3,8,9,11} However, pH measurements cannot detect GOR in the pH range 4.0–7.0 due to the proximity to the normal oesophageal pH. Consequently, pHmetry misses many episodes of post-prandial reflux in infants because of neutralization of gastric contents by milk formula for 1 to 2 hours after a meal as it simply measures the H⁺ ion concentration at the sensor site. Therefore the terms “acid”, “neutral”, or “alkaline” “GOR” should be preferred to the blanket term “GOR.” Because the occurrence of GOR-associated symptoms may be independent of pH, a pH-independent technique that facilitates an ambulant 8- to 24-hour measurement for GOR detection has been established and relies on differing conductivities of luminal contents. This is termed *multiple intra-luminal impedance/pH* and has been previously described in detail.¹²⁻¹⁴ This allows, for the first time, the physiologically-appropriate assessment of treatment efficacy for GOR.

The patho-physiological mechanism hypothetically invoked to explain cow's milk protein-associated upper gastrointestinal (GI) allergy and consequently presumed GI dysmotility, that of the clinical entity of cows' milk associated reflux oesophagitis, is presumed to include gastroparesis and lower oesophageal relaxation episodes (LOS), leading to symptoms of reflux such as post-prandial pain and feed-associated irritability.

This variant of cow's milk allergy appears to be a particularly common manifestation in infancy, with symptoms indistinguishable from primary gastro-oesophageal reflux but settling on an allergen exclusion diet.⁴ Some differentiation from primary reflux has been suggested on the basis of an oesophageal pH-testing pattern and β lactoglobulin antibody response.^{15,16} Recent murine studies showed a pathway in which enteric antigen induces an eotaxin and IL-5 dependent recruitment of eosinophils and TH2 T cells in sensitized animals, which disrupt enteric neurones to induce dysmotility.¹⁷ The motility disturbance of cow's milk sensitive-associated reflux may thus occur as a neurological consequence of inflammatory infiltration induced from lamina propria into the epithelial compartment.¹⁷

Although it is now clear that multiple food antigens may induce oesophagitis,^{18,19} the most common precipitant is cow's milk protein. Standard endoscopic biopsy and histology do not reliably distinguish between primary reflux oesophagitis and the emerging clinical entity of cow's milk sensitive reflux oesophagitis (CMSRO). This variant of cow's milk allergy appears to be a particularly common manifestation in infancy, with symptoms indistinguishable from primary gastro-oesophageal reflux, but settling on an exclusion diet. This suggests the possibility of a distinct mechanism in CMSE, in which mucosal homing to the oesophagus occurs of lymphocytes activated within the small intestine. This may explain the seemingly counter-intuitive finding of basal, as opposed to superficial, chemokine expression and the common occurrence of mucosal eosinophilia in this condition.²⁰ The oesophageal motility disturbances of CMSRO are thus postulated to occur as a neurological consequence of the inflammatory infiltration induced from lamina propria vessels into the epithelial compartment.²¹ This proposed mechanism contrasts with the current concept of lumenally-induced inflammation found in primary reflux oesophagitis, and is consistent with the characteristic delayed onset and chronic nature of cow's milk-associated reflux oesophagitis.

MATERIALS AND METHODS

The study included 11 patients (6 male) of median age 151 days (range 75–345). Patients were eligible if they were under 12 months of age, had symptoms clinically suggestive of GOR (e.g. regurgitation

>3x/day any amount or > once/day half the feed), were over 2000g in weight, and were exclusively bottle fed formula milk or expressed breast milk and had no signs of acute infection. A clinical suspicion of GI-related cow's milk protein allergy was a mandatory inclusion criterion. Patients who were taking acid suppressing or motility agents had therapy stopped at least 3 days (5 days in the case of omeprazole) before beginning the study.

The study protocol was approved by the Royal Free NHS Trust Ethical Review Committee, with informed parental/guardian consent obtained. On the day of simultaneous IMP/pH measurement, patients were fasted for a period of at least 3 hours. An IMP catheter (diameter 2 mm) with two pH-sensitive antimony electrodes and seven impedance electrodes (Sandhill Scientific, Inc) was used (Figure 1). Changes in intra-oesophageal impedance were measured along this catheter as described in previous work.¹²⁻¹⁴ The catheter was placed trans-nasally and positioned using the Strobel formula,²² with total measuring segments reaching from approximately 1.5 cm above the lower oesophageal sphincter (channel 6) to the upper oesophagus (channel 1), both of which also had pH sensors. The beginning and end times of feeding periods were entered on screen in real time and recordings were made for 24 hours. During this 24-hour period, the infants were fed on the first occasion with their standard milk and 14 days after the substitution of this cow's milk-based formula with the amino acid-based formula Neocate®. The 24-hour study was repeated, with all other parameters remaining the same as closely as possible (e.g., the amount of time during the post-prandial period during which the infant was in various body postures, and the amount of time lying supine in sleep, and the number and volume of feeds—no statistically significant difference was seen between these parameters between the two feeding periods). No patient received any anti-reflux medications before or during the study period.

A list of criteria was devised in order to delineate infants with a well-defined increased risk of developing allergic disease. This included the European Academy of Allergy and Clinical Immunology (EAACI) criteria for such infants—i.e., those with at least one first-degree relative (parent or sibling) with docu-

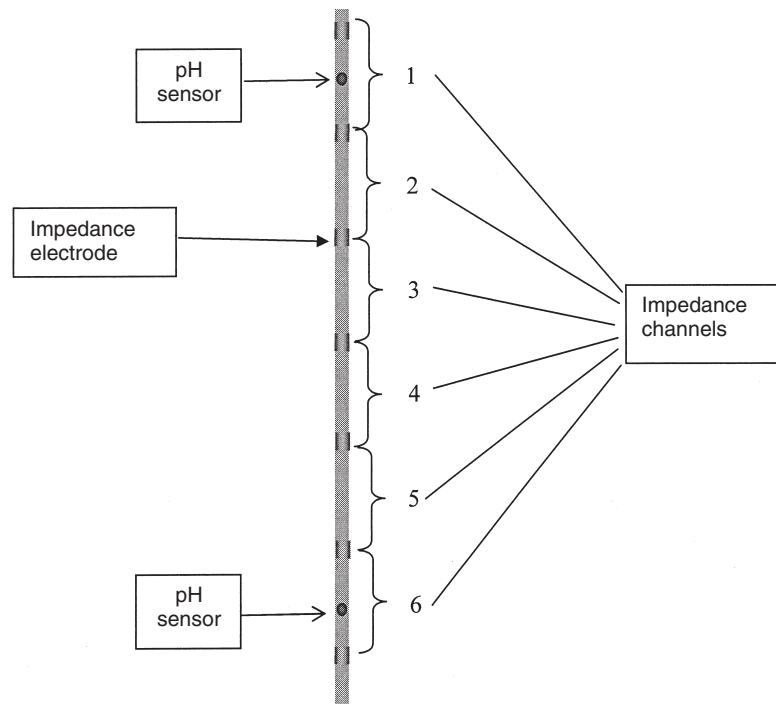


FIG. 1. Schematic representation of the impedance catheter with 7 impedance electrodes set at 1.5 cm intervals and comprising 6 measuring segments (impedance channels). Distal and proximal pH electrodes were also incorporated to detect the pH of the refluxate at approximately 2 cm above the LOS and in the upper oesophagus.

mented allergic disease²³ coupled with objective criteria based on the child's clinical phenotype and allergy tests. Blood samples from all children were subjected to food-specific immunoglobulin E (IgE) assays (milk, egg, soya, wheat, and peanut) as well as total IgE. All IgE assays were performed within the same laboratory (Royal Free Hospital, London) using the CAP system FEIA technique (Pharmacia & Upjohn, Uppsala, Sweden). Skin Prick Tests were performed on healthy skin on the volar forearm with commercially available cow's milk, egg, soya, wheat, and peanut allergen extracts (ALK, Horsholm, Denmark). Reactions were read after 15 minutes, and a response was recorded as positive if the mean wheal diameter was 3 mm or greater in combination with a negative control of 0mm. An atopy patch test was performed using filter paper soaked in fresh cow's milk and 12 mm aluminium test cups (Finn Chamber, Epitest Ltd, Hyryla, Finland) attached to healthy skin of the back with Scanpore tape. The occlusion time of patch testing was 48 hours. The results were read 15 minutes after removal of the patch with a final reading at 72 hours after initial patch application. Final reactions were considered to be positive if, according to previously published recommendations²⁴, erythema and clear infiltration occurred. Any child who fulfilled one or more criteria was considered to be at a high risk of suffering from allergic disease.

For the 24 hours of the feeding period for each feed, a standardized and validated infant GOR symptom score was completed by the parent or carer.²⁵ (Table 1)

All impedance recordings were visually analyzed, blind to type of feed used, for the typical IMP pattern of GOR indicated by a retrograde oesophageal volume flow (Figure 2). All GOR episodes were subdivided by their pH values. In addition, the distribution of GOR episodes was determined for each measuring period, defined by post-prandial time. The height reached by the refluxate in the oesophagus, along with volume- and acidity-clearance, were registered for each GOR episode. Data sets for each patient before and during the use of Neocate® were compared. The following parameters were derived from the pH and impedance traces:

- study duration
- number of reflux events (and number of reflux events per hour)
- number of non-acid reflux events (and number of non-acid reflux events per hour)
- number of acid (pH < 4) reflux events (and number of acid reflux events per hour)

TABLE 1. INFANT GASTRO-OESOPHAGEAL REFLUX QUESTIONNAIRE

<i>Question</i>	<i>Points</i>
How often does the baby usually posset/vomit?	
1 to 3 times a day	1
3 to 5 times a day	2
More than 5 times a day	3
How much does the baby usually posset/vomit up?	
A teaspoonful to a tablespoonful	1
A tablespoonful to an ounce	2
An ounce or more	3
Does the possetting/vomiting seem to be uncomfortable for the baby?	2
Does the baby refuse feeds even when hungry?	1
Does the baby have trouble gaining weight?	1
Does the baby cry a lot during or after feeds?	3
Do you think the baby cries or fusses more than normal?	1
How many hours does the baby cry or fuss each day?	
1 to 3 hours	1
More than 3 hours	2
Do you think the baby hiccups more than most babies?	1
Does the baby have spells of arching of the back?	2
Has the baby every stopped breathing while awake, struggled to breathe, or turned blue or purple?	6
Total	25

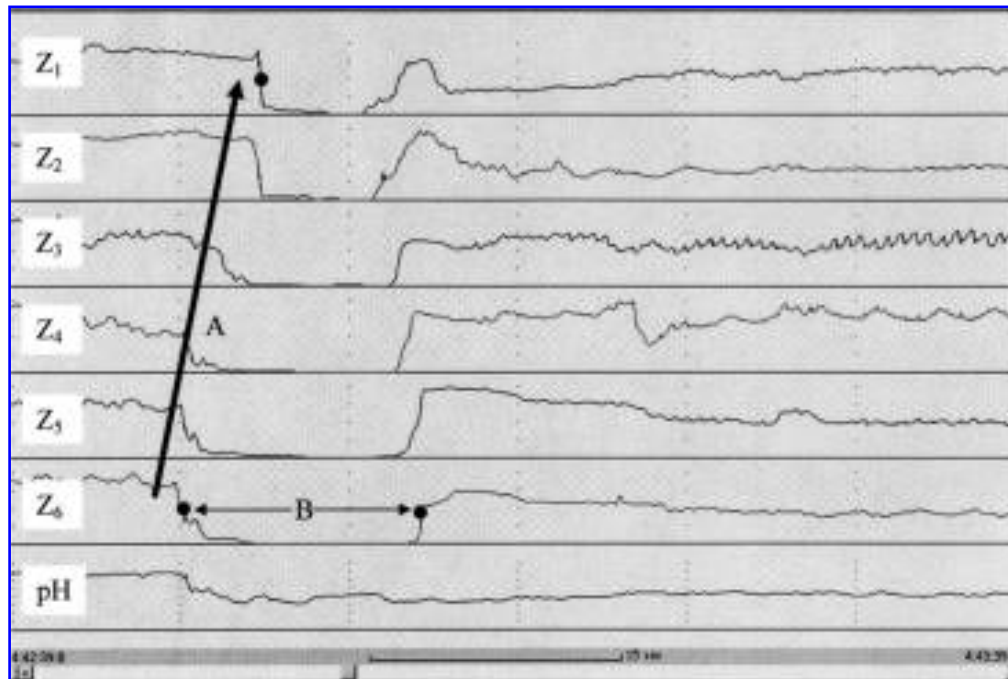


FIG. 2. Impedance pattern typically observed during a reflux episode. In this example, only the distal pH channel is shown, which is located approximately 1.5 cm above the LOS. The retrograde oesophageal flow is indicated by arrow A. In this example the reflux reaches the proximal (highest, Z_1) channel. The reflux duration is indicated by arrow B. The pH of the reflux can be derived from the pH channel, and the acid clearance is the time taken for the pH channel wave to reach pH 4.

- number of reflux events in the first 2 hours after the feed
- average reflux height (based on the “height” (the highest impedance electrode) at which each reflux event was detectable)
- average minimum distal pH
- average minimum proximal pH
- total acid clearance time per hour (total time with distal pH below pH 4 relative to study duration) for acid reflux events
- total reflux duration time per hour (based on the impedance traces)

Parameters were summarized for each milk ingestion episode, and the differences between treatments were assessed using the Wilcoxon signed rank test. The number of reflux events per hour was compared between the period consisting of the first two hours after feeding for each feed and the residual period until the next feed. Data for each treatment were assessed independently.

RESULTS

Table 2 summarizes the main impedance/pH results by milk when on the standard milk ingested and Neocate®. A total of 747 reflux events were detected by IMP, 518 of which were non-acid and 229 were acidic (pH < 4). The median number of reflux events per hour, median number of acid reflux events per hour, and median number of reflux events during hours 1 or 2 were not significantly lower on Neocate® than when on the standard milk formula. In addition, average reflux height and average minimum distal or proximal pH was not different.

On the basis of our criteria, 8 of the 11 children were considered at high risk of allergic disease (Table 3).

TABLE 2. SUMMARY OF STANDARD MILK VERSUS NEOCATE IMPEDANCE/pH DIFFERENCES

<i>Parameter</i>	<i>Standard milk</i>	<i>Neocate</i>	<i>Difference (Standard milk– Neocate)</i>	<i>p-value^a</i>
Number of reflux events per hour:				
median	1.96	2.25	–0.29	0.67
range	(0.38–3.54)	(0.79–4.71)	(–1.33–+1.25)	
Number of acid reflux events per hour:				
median	1.04	1.25	0.20	0.50
range	(0.04–2.42)	(0.25–3.58)	(–2.04–+1.92)	
Reflux Index:				
median	1	1	0	0.35
range	(0.2–15.2)	(0.4–13.4)	(–11.0–+13.7)	
Number of reflux events in post-feed hours 1 or 2:				
median	1.54	1.04	0.50	0.15
range	(0.33–2.42)	(0.50–2.54)	(–0.71–+1.38)	
Average reflux height ^b :				
median	2.0	2.0	0	0.53
range	(1.05–2.58)	(1.03–3.37)	(–1.85–+1.0)	
Average minimum distal pH:				
median	4.0	4.0	0	0.89
range	(3.32–6.42)	(2.82–5.61)	(–2.07–+2.42)	0.411
Bolus clearance time (sec):				
median	12.0	12.0	0	0.32
range	(8.42–27.8)	(7.36–36.0)	(–8.2–+3.98)	0.225
Acid clearance time (sec):				
median	46.0	58.0	–12.0	0.40
range	(14.1–195.0)	(27.2–107.8)	(–45.0–+87.25)	0.322

^ap-values from Wilcoxon signed rank test.

^bPharynx is denoted as 1.0, whereas 1 cm above gastro-oesophageal junction is denoted as 6.0 (i.e., lower numeral denotes higher bolus passage from stomach proximally up the oesophagus).

All of these children had a positive family history of allergy in a first-degree relative, while 4 of these also fulfilled a further criterion, such as personal history of eczema (all 4 children) or allergy test positivity to a common food allergen (3 of 4). The subjective but semi-quantitative parent/caregiver reflux questionnaire revealed a significant improvement on Neocate[®] compared to their standard formula in these 8 infants of 15(12–18) to 9(5–11) (median (range)) ($p = 0.0019$). This is in contrast to the 3 infants who did not fulfill any of our atopic criteria. In these children, who were considered to be at low risk of developing food allergy, the change of 12(11–15) to 12(4–12) was not statistically significant. Thus, the differences were more marked in the more atopic individuals, although perhaps, due to small numbers, this did not reach statistical significance for the non-atopic group. Nevertheless, it is interesting to note that while the GORD scores improved in the atopic group, it was not paralleled by an improvement in the objectively measurable reflux as assessed by combined intra-luminal impedance and pH.

DISCUSSION

We have described an interventional cohort study that was prospective—but not blinded—to investigate the potential influence of Neocate[®] on apparent GOR-type symptoms in infants by using the novel and

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TABLE 3. ALLERGIC STATUS OF PATIENTS

Patient	Family history	Personal history of atopy	Total serum IgE	Specific IgE	Skin prick test	Atopy patch test
1	0	0	<100 kU/litre	All negative	All negative	All negative
2	0	0	<100 kU/litre	All negative	All negative	All negative
3	1	0	<100 kU/litre	All negative	All negative	All negative
4	1	Eczema	<100 kU/litre	10 kU/l to peanut	4 mm to peanut	All negative
5	0	0	<100 kU/litre	All negative	All negative	All negative
6	1	Eczema & food allergy	<100 kU/litre	7 kU/l to egg	3 mm to egg	All negative
7	1	0	<100 kU/litre	All negative	All negative	All negative
8	1	Eczema	<100 kU/litre	All negative	All negative	All negative
9	1	0	<100 kU/litre	All negative	All negative	All negative
10	1	Eczema	<100 kU/litre	12 kU/l to peanut	4 mm to peanut	All negative
11	1	0	<100 kU/litre	All negative	All negative	All negative

physiologically appropriate technique of combined pH and intraluminal impedance measurement. Although the parents/caregivers perceived a symptomatic improvement as documented by the semi-quantitative GOR symptom questionnaire, there was no observable difference in any reflux parameter as measured by this new more physiologically appropriate method. There may have been 3 reasons for this: first, there was no effect of Neocate® on reflux in infants and the symptomatic difference is due to parental placebo effect; second, there was no effect of Neocate® on reflux in infants and the effect is due to another physiological effect of the use of Neocate® such as a hypothetical improvement in small bowel dysmotility, however this is less likely as the GORD symptom score decreased in the majority of the infants; or third, the study did not include a sufficient numbers of infants to produce a statistically significant difference in reflux, while having numbers sufficient enough to observe a difference in symptoms (or again evoking the parental/caregiver placebo effect). Thus, it may be argued that larger studies are needed and should be placebo-controlled, although this is ethically questionable and practically difficult because Neocate® has a noticeable difference in odor from normal cow's milk-based formulas—parents/carers would undoubtedly notice this and the infants, at least at first, may exhibit a degree of feeding reluctance. There was a trend toward greater GORD symptomatic improvement in those considered more likely to have food allergies in all 8 infants when on Neocate®, but there was no significant improvement in symptoms traditionally attributable to reflux in 2 out of 3 of those not fulfilling such atopic criteria. In contrast, while 4 of 7 at higher risk of food allergy did improve, 2 of 7, did not.

The technique of combined pH and impedance procedure allows detection of both acid and non-acid reflux episodes, the latter therefore undetectable by conventional pHmetry. In our study, we identified 747 reflux episodes of which 518 (69.3%) were non-acid, i.e., the vast majority of 747 reflux events detected by impedance would not have been detected by the so-called “Gold-Standard” (i.e., pHmetry), which only identified 229 of 747 (30.7%). Wenzl et al.¹³ found that 78% of reflux episodes causing apnoea in infants were non-acid, and showed that out of 1887 IMP-determined reflux episodes recorded in 50 patients only 282 (14.9%) were acidic.¹⁴

In conclusion, the physiologically appropriate denominator (combined intra-luminal impedance/pH) has been used in this study to objectively study in a case-controlled way the effect of Neocate® on gastro-oesophageal reflux and symptoms traditionally attributed to infant reflux. The findings do not suggest a significant effect of this formula on measurable reflux in this population, but there is a decrease in parentally-reported subjective semi-quantitated reflux symptom score in the atopic group more than the non-atopic group. Any observed clinical effect of this preparation must therefore be assumed to be either of a placebo nature or due to a different physiological mechanism such as the hypothetical improvement in colic-like pain due to improved small bowel motility linked to cow's milk GI sensitisation, or perhaps attendant oesophageal spasm causing pain, which would not be shown by any improvement in measured reflux parameters.

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