Title: The impact of compliance during exclusive enteral nutrition on faecal calprotectin in children with Crohn's disease

Shona Mckirdy[1], Richard K. Russell[2], Vaios Svolos[1], Konstantinos Gkikas[1], Michael Logan[1], Richard Hansen[3] Konstantinos Gerasimidis[1]*

[1]Human Nutrition, School of Medicine, Dentistry and Life Sciences, University of Glasgow, New Lister Building, Glasgow Royal Infirmary, G31 2ER, Glasgow, UK

[2]Department of Paediatric Gastroenterology, Royal Hospital for Children & Young People, Edinburgh EH16 4TJ , UK

[3]Department of Paediatric Gastroenterology, Royal Hospital for Children, Glasgow G51 4TF, UK

*Corresponding author

Human Nutrition, School of Medicine, Dentistry, and Nursing, College of Medical, Veterinary and Life Sciences, University of Glasgow, New Lister Building, Glasgow Royal Infirmary, G31 2ER, Glasgow, UK

Tel: 0044 141 201 8689

Email: Konstantinos.Gerasimidis@glasgow.ac.uk

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Author Contribution

Shona Mckirdy: Performed statistical analysis and drafted the manuscript; Richard K. Russell:

Identified and recruited patients for the study and revised the draft manuscript; Vaios Svolos and

Konstantinos Gkikas: Performed laboratory analysis; Michael Logan: Collected the samples and data;

Richard Hansen: Identified and recruited patients for the study and revised the draft manuscript;

Konstantinos Gerasimidis: Conceived and co-ordinated the study, revised the draft manuscript, and

supervised all research activities.

ABSTRACT

It remains unclear whether suboptimal response to exclusive enteral nutrition (EEN) in some children with Crohn's disease (CD) is explained by poor compliance. The current study measured faecal gluten immunogenic peptides (GIP), a biomarker of gluten intake, in 45 children (3-17 years) with CD, and explored associations with faecal calprotectin (FC) levels at 33 and 54 days of EEN. FC decreased in patients with undetectable GIP at both 33 and 54 days of EEN (mean decrease, 33 days: -743 mg/kg, 54 days: -1043 mg/kg, p<0.001), but not in patients who had detectable levels. At EEN completion, patients with undetectable GIP had a lower FC by 717 mg/kg compared with patients with a positive GIP result (p=0.042) and demonstrated a greater decline from baseline FC (-69% vs +5%, p=0.011). Poorer response to EEN is explained in part by diminished compliance. Faecal GIP might be useful as proxy biomarker of EEN compliance.

What is known:

- Exclusive enteral nutrition is an effective treatment for active Crohn's disease in children.

- The extent to which lack of, or diminished responses to EEN is explained by poor treatment compliance remains unclear due to the absence of objective biomarkers.

What is new:

- A statistically significant decrease in faecal calprotectin levels was observed only in patients who had absence of gluten immunogenic peptide in faeces throughout EEN.

- Gluten immunogenic peptide might be useful as biomarker to ascertain adherence to EEN in addition to dietetic/clinical assessment.

INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory condition of the gut. Exclusive enteral nutrition (EEN) is the first-line treatment for active paediatric CD, achieving remission rates of up to 80% (1) while concomitantly decreasing faecal calprotectin (FC) concentration, an objective and clinically established marker of gut inflammation (2). However, very few patients achieve FC levels within the normal range by the end of the treatment despite improvement in clinical disease activity (2, 3). Palatability and the restrictive nature of EEN are limiting factors of its use; hence, compliance to the treatment remains a major challenge to some patients (4). Poor adherence is also reported as a major reason why clinicians in North America are less likely to use EEN as a treatment for CD (5, 6).

Currently, there are no objective ways to assess compliance to EEN in routine clinical practice and it is also unclear to what extent this influences treatment outcome. All EEN formulas used for the management of CD are gluten-free (7). Hence, we postulated that presence of gluten immunogenic peptides (GIP) in faeces, a biomarker of gluten consumption (8), would indicate a group of patients more likely to deviate from EEN and allow us to study the extent to which this may explain lack of or diminished FC responses to treatment with EEN.

This study investigated the relationship between GIP in faeces and FC responses in paediatric patients with CD undergoing an 8-week induction course with EEN.

METHODS

The present study included data and analysed samples from paediatric patients with CD recruited from the Royal Hospital for Children, Glasgow between 2014-2017. Clinical aspects from this patient cohort in response to EEN therapy have been published previously (3). Both newly diagnosed patients undergoing their first course of EEN and those on a subsequent course after disease exacerbation were recruited. Patients were only included in this present study if they were deemed fully compliant to the 8-week course of EEN via clinical dietetic review.

Faecal samples were collected at baseline, 33 and 54 days during treatment with EEN. FC and GIP levels were measured using the CALPROLAB0170 (ALP) (Lysaker, Norway) ELISA kit and the iVYDAL *In Vitro* Diagnostics iVYLISA GIP-S kit (Biomedal S L., Seville, Spain), respectively, following manufacturer instructions. Data on inflammatory markers of patients including serum albumin, plasma C-reactive protein and erythrocyte sedimentation rate were collected from patient notes.

Faecal GIP concentrations were classed in a binary manner. Patients with undetectable GIP concentration were categorised as "Negative", while patients with detectable concentration were classed as "Positive". Differences in FC concentrations and clinical data between groups or across the timepoints in each subject were assessed with a general linear model and Box-Cox transformation; pairwise differences were reported with Tukey post-hoc test. The proportion of FC variance explain by GIP levels was tested with the general lineal model. A p-value of <0.05 was considered statistically significant. Statistical analysis was performed with MINITAB® 19.2020.1, Coventry UK. The study was approved by the NHS West of Scotland Research Ethics Committee (14/WS/1004) and was registered in clinicaltrials.gov (NCT02341248).

RESULTS

Forty-five patients were included in the present study (Table 1). Twenty-seven (60%)

patients had data collected for all three timepoints while the remaining patients had data

for at least two. Two samples at the final timepoint of EEN were excluded from analysis as

they were collected three and six days after EEN completion, when patients had

reintroduced their habitual diet. These samples were excluded as detectable GIP in samples

collected ≥2 days post-EEN would likely reflect reintroduction of habitual diet rather than

poor compliance to EEN (9). Prior to EEN, 37/40 patients (93%) who provided faecal samples

were GIP positive, indicating gluten consumption in their habitual diet. One of the patients

with GIP negative samples at baseline had a concomitant diagnosis of coeliac disease and

was following a gluten-free diet. Thirteen and 23% of patients had detectable levels of GIP

at 33 and 54 days of EEN indicating non-compliance (Table 1). By the end of EEN, 35 patients

(78%) entered clinical remission (weighted paediatric Crohn's disease activity index ≤12.5).

Of the 10 patients who did not achieve clinical remission, GIP at 54 days of EEN was

measured in seven, three of whom had detectable levels.

During EEN, mean FC decreased significantly from levels at treatment initiation, in

patients who were GIP negative at both 33 and 54 days of EEN (mean, SD treatment

initiation: 1491, 548 g/kg; 33 days: 895, 617 g/kg; 54 days: 648, 669 g/kg, both $p<0.001$,

n=25). In contrast, in patients who were GIP positive at either 33 or 54 days, FC

concentration did not change from levels at treatment initiation (mean, SD treatment

initiation: 1287, 425 g/kg; 33 days: 998, 526 g/kg; 54 days: 993, 637 g/kg, $p=0.282$, n=10). In

the five patients who were GIP negative at 33 days but subsequently became GIP positive at

54 days of EEN, there was no difference in FC levels between these two timepoints (mean,

SD 33 days: 1202, 649 g/kg vs 54 days: 1021, 510 g/kg $p=0.759$). In contrast, 20 patients who

tested GIP negative at both 33 days and 54 days of EEN showed a significant decrease in FC levels (mean, SD 33 days: 918, 644 g/kg vs 54 days: 698, 714 g/kg respectively, p=0.02).

In cross-sectional analysis, there was no difference in FC concentration between patients who had positive and negative GIP both at treatment initiation (mean, SD GIP positive: 1456, 532 g/kg vs GIP negative: 1383, 569 g/kg, p=0.8) as well as at 33 days of EEN (mean, SD GIP positive: 794, 314 g/kg vs GIP negative: 940, 622 g/kg, p=0.61) (Figure 1). However, patients who were GIP negative at 54 days of EEN had significantly lower mean compared with those who were GIP positive (mean, SD GIP Positive: 1152, 611 g/kg vs GIP negative: 627, 648 g/kg, p=0.042) (Figure 1).

Similarly, at 33 days of EEN, there was no difference in the percentage change of FC from treatment initiation between patients with negative (n=29) or positive GIP (n=5) (p=0.84). However, at 54 days of EEN, patients who were GIP negative showed a larger decrease in FC from treatment initiation compared with patients who were GIP positive (-69% vs +5%, p=0.01, Figure 1).There were no differences in CRP (p=0.434) or serum albumin levels (p=0.60) observed between patients with and without detectable levels of GIP in faeces. In regression analysis, the absolute concentration of GIP was a significant predictor of FC levels at 54 days of EEN (R^2=9.5%, p=0.040).

TABLE 1 HERE

FIGURE 1 HERE

DISCUSSION

To our knowledge, this is the first study to investigate the relationship between non-compliance to EEN and FC response in paediatric CD using objective biomarkers of treatment. In patients with undetectable GIP during EEN, FC showed a continual decline from baseline, while no change was detected in patients who were GIP positive at either 33 or 54 days of EEN. In patients who were GIP negative at 33 days but subsequently became GIP positive at 54 days, FC levels also did not change, indicating that eventual non-compliance may negatively affect treatment efficacy where FC would otherwise continue to decrease. The absence of difference between the two groups at 33 days of EEN may be due to low statistical power or that dietary transgressions to EEN were more common during the second half of the treatment. Furthermore, patients who were GIP negative at 54 days on EEN had significantly lower FC than those who were GIP positive and had a substantially larger proportional drop in FC from baseline. These findings support the concept that non-exclusive compliance to a course of EEN influences response to treatment in patients with CD and supports the premise that exclusivity with complete withdrawal of table food is generally needed for optimal treatment outcomes, including promotion of mucosal healing (10). However, while GIP detection might negatively influence FC response, the same was not observed for ESR, CRP, or serum albumin which may be less senstive to short term changes in gut inflammation with dietary transgression during EEN.

Gluten is a ubiquitous and often unavoidable ingredient in our diet, often used as an additive in the food industry. Approximately 5-15 grams of gluten is consumed per day in a typical Western diet, and it may even be accidentally consumed by patients with coeliac disease actively trying to exclude it from their diet (9). This makes detection of GIP in faeces

a plausible biomarker of compliance during EEN; particularly as GIP is detectable in individuals who have a very low intake of gluten in their diet (9). There are, however, limitations to this approach, since detection of GIP in faeces is only indicative of short-term consumption of gluten-containing foods and can appear in stool 1-2 days after gluten consumption depending on transit time (8, 9). Therefore, in patients who consumed gluten-containing food outwith this timeframe, faecal GIP will not be detected. Moreover, non-compliance due to consumption of other gluten-free foods was not assessed in this study which is a limitation of using GIP as a sole biomarker of compliance to EEN. This may also explain why some patients with negative GIP in faeces still had high levels of FC at the end of EEN, and in addition to the proportion of patients who are not expected to response to EEN, despite treatment adherence. Further work is needed to explore other dietary indiscretions during EEN, how these can be identified and quantified, and how these impact on the success of therapy. Based on the data we present here, >95% of patients had consumed gluten in their habitual diet prior to EEN initiation. The upper limit of detection for GIP concentration of the kit used in this study was 1250 ng/g of faeces. This may roughly equate to intake of around 1.5-2.5 g of gluten (9) which can be found in 25 g of wheat flour (11) or a 30 g slice of wheat bread (12). By itself, this makes GIP a very sensitive biomarker of even small dietary transgression with gluten-containing food during EEN.

This pilot study is limited by small sample sizes and few incomplete sample series. Future independent research should replicate the findings of this study.

In conclusion, this study suggests that non-compliance may have a negative influence over FC response during EEN in paediatric CD, and that GIP might be a useful biomarker to objectively assess adherence in addition to clinical dietetic review.

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FIGURE LEGENDS

Figure 1: Individual value plots of faecal calprotectin concentration (A) and % change faecal calprotectin from treatment initiation (B) at 33 and 54 days of exclusive enteral nutrition stratified by negative and positive faecal gluten immunogenic peptide (GIP). Black horizontal bars represent the median.
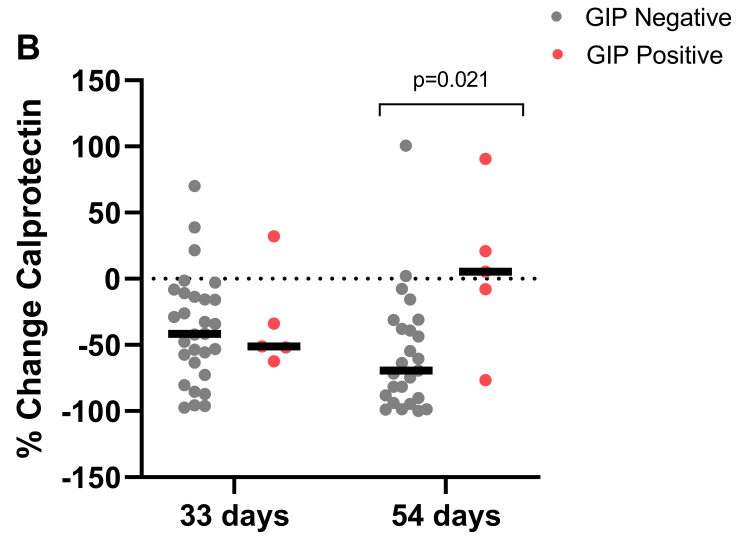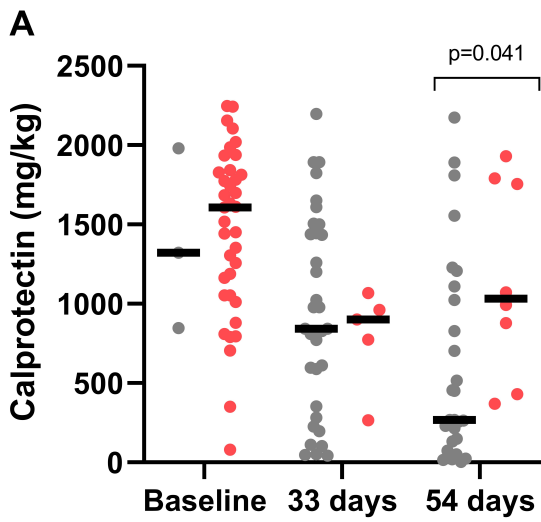
Table 1: Participant characteristics and clinical data across the three timepoints

		Baseline		33 days		54 days
	n	*Mean (SD)*	*n*	*Mean (SD)*	*n*	*Mean (SD)*
Age, Mean (SD) years	45	12.3 (3.08)	45	12.3 (3.08)	45	12.3 (3.08)
Height z-score, Mean (SD)	45	-0.1 (0.92)	27	-0.19 (0.95)	40	-0.15 (0.88)
BMI z-score, Mean (SD)	45	-0.56 (1.22)	28	-0.41 (1.02)	40	0.13 (0.77)
FC, Mean (SD) mg/kg	40	1450 (527)	39	921 (590)	35	747 (669)
wPCDAI, Mean (SD)	45	41.2 (22.2)		N/A	45	8.61 (10.6)
Males (%)	45	67	45	67	45	67
Short Stature (%)	45	0	25	4	30	0
Underweight (%)	45	10	26	4	30	0
Raised ESR (%)	37	46		N/A	30	30
Low Albumin (%)	44	48		N/A	30	7
Raised CRP (%)	41	51		N/A	27	18
Detected GIP (%)	40	93	39	13	35	23

Short Stature defined as height z-score <-2; Underweight defined as BMI z-score <-2; Raised ESR defined as >20 mm/hr; Low albumin defined as <35 g/L; Raised CRP defined as >7 mg/L; wPCDAI= weighted paediatric Crohn's disease activity index, ESR= erythrocyte sedimentation rate, CRP= C-reactive protein, GIP= gluten immunogenic peptides.