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Posted Date: 24 May 2024

doi: 10.20944/preprints202405.1632.v1

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Short Communication

Comparative Performance of Stool Gluten Immunogenic Peptide Platforms for Monitoring Adherence to the Gluten-Free Diet

Juan P. Stefanolo [1], Roberto Puebla [1], Martín Tálamo [1], Samantha Dodds [1], María P. Temprano [1], María E. Oregui [1], María L. Espinet [1], Edgardo Smecuol [1], Sonia I. Niveloni [1] and Julio C. Bai [1,2]

[1] Teaching and Research Department; Dr. C. Bonorino Udaondo Gastroenterology Hospital. Buenos Aires; Argentina.
[2] School of Medicine, Research Institutes; Universidad del Salvador. Buenos Aires; Argentina.
* Correspondence: jbai@intramed.net; Teaching and Research Department; Dr. C. Bonorino Udaondo Gastroenterology Hospital, Av. Caseros 2061; (1264) Buenos Aires; Argentina; Telephone: +5411-4304-1018; Fax: +5411-4304-1018

Abstract: Summary: Background/Aim: Stool gluten immunogenic peptides (GIP) determination is now considered a valuable tool for detecting dietary lapses in treated celiac disease (CeD) patients. Our study aims to explore the diagnostic performance of a qualitative lateral flow immunoassay point-of-care test (LFIA/PoCT) compared with a qualitative enzyme-linked immunosorbent assay (ELISA) for detection of stool GIP. Methods: The study is a post hoc analysis of stool samples collected in the real-life conditions from a cohort of gluten-free diet-treated CeD patients. Stool GIP was measured in μg/g of stool using an ELISA kit (iVYLISA GIP S®, Biomedal SL). For the qualitative evaluation of stool GIP, a LFIA/PoCT test (GlutenDetect®; Biomedal S.L) was employed, which reported results as positive or negative. The LFIA/PoCT performance was evaluated against ELISA, considered as the gold standard. GIP concentrations <0.156 μg/g were deemed negative, while ≥0.64 μg/g were considered capable of producing intestinal damage. Results: Fifty-three patients collected a total of 293 stool samples. Among these samples, 115 (39.2%) tested positive for ELISA (≥0.156 μg/g). At this cutoff, LFIA/PoCT exhibited a sensitivity of 46.1% and a specificity of 88.8%, with positive and negative predictive values of 72.6% and 71.8%, respectively. Regarding, the ≥0.64 μg/g cutoff (n=65), LFIA/PoCT improved sensitivity (55.4%), albeit with a slightly reduced specificity (83.8%), and positive and negative predictive values of 49.3% and 86.8%, respectively. The agreement coefficient between ELISA and LFIA/PoCT tests was 78.0% for concentrations ≥0.64 μg/g. Conclusion: Despite being less sensitive than the ELISA platform, the LFIA/PoCT stool GIP test provides a straightforward and convenient option for patients to self-monitor adherence to the gluten-free diet.

Keywords: Celiac disease; follow-up; stool gluten immunogenic peptides; gluten exposure; gluten-free diet

1. Introduction

Celiac disease (CeD) is an autoimmune disorder characterized by a T-cell-mediated reaction to gluten, a protein found in wheat, rye, and barley, affecting genetically predisposed individuals1. Currently, the only recognized treatment for CeD is a lifelong strict gluten-free diet (GFD) [1,2]. However, maintaining strict dietary adherence presents significant challenges, and patients frequently experience voluntary or involuntary gluten exposures3. Various tools are employed to assess adherence to the GFD, but they have limitations, and cannot be easily employed for health providers and patients.

Recently, the emergence of quantitative and qualitative tests for detecting gluten immunogenic peptides (GIP) in stool has provided valuable tools for evaluating dietary gluten exposure [4–6]. These tests utilize quantitative enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassay (LFIA) platforms, with the latter available for qualitative point-of-care testing

 doi:10.20944/preprints202405.1632.v1

(LFIA/PoCT) in clinical settings [7]. While the ELISA platform has been considered the gold standard for testing stool GIP, it requires the collection and transportation of samples to a dedicated laboratory, imposing costs and burdens on both patients and healthcare providers [8,9].

In this study, our aim was to evaluate the performance of a stool LFIA/PoCT test in comparison with the quantitative determination of stool GIP using ELISA in order to assess the accuracy and reliability of the last in detecting gluten exposure in a real-life setting.

2. Materials and Methods

Study Design

This post hoc analysis involved systematically and prospectively collecting a series of stool samples from a cohort of 53 treated CeD patients [10]. The aim was to compare the performance of the LFIA/PoCT with the ELISA method in the same set of samples. The objective was to assess whether the rapid qualitative home test provides a reliable and convenient alternative for patients to monitor adherence to a GFD. Celiac disease-specific serology was determined at entry the study by using IgA tissue transglutaminase (IgA tTG) antibodies and IgA deamidated gliadin (IgA DGP) antibodies. Presence of symptomatic CeD was determined according to a celiac symptom index (CSI) ≥38 points [10].

Population Screening

Inclusion and exclusion criteria: All patients were screened and enrolled at the Celiac Disease Clinic of the Dr. C. Bonorino Udaondo Gastroenterology Hospital in Buenos Aires. Adult CeD patients were enrolled if meeting the following criteria: (1) a well-established histological (type 3 Marsh) and serologic diagnosis of CeD [11]; (2) a self-estimation of adherence to the GFD for more than 2 years; (3) having at least 1 stool/day or every other day; (4) collect and keep samples frozen, and transport them to our institution in special containers. Patients who were unwilling to participate, had uncontrolled concomitant clinical disorders (e.g., type I diabetes, hypothyroidism, etc.), type II refractory CeD, or were taking drugs that could potentially affect stool GIP were excluded of the study.

Gluten Immunogenic Peptides Stool Tests and Procedures

Patients were instructed to collect two sets of 2-4 g of stool from the first morning deposition and immediately place them into sealed containers, which were then frozen until transportation to the lab [10]. Stool samples remained frozen until ELISA tests and LFIA/PoCT were performed. Stool samples were assessed for GIP levels using the iVYLISA GIP-S kit (Biomedal S.L., Sevilla, Spain), which detects gluten peptides surviving digestion and reaching stools, with a reported detection limit ≥0.156 μg/g. Point-of-care testing for stool GIP was conducted in our lab according to indications by experts (RP and SD) who were blind to the ELISA results. The LFIA/PoCT tests for stool GIP were categorized as negative or positive based on visual interpretation of LFIA/PoCT results [7]. Details regarding detection limits, statistical performance of tests, time lapses from consumption to detection, and clearance times for stool and urine have been previously reported [7]. The immunochromatographic strip was examined and read after 10 minutes by the same experts for the presence of a red line in the test area, indicating a positive result. Operators remained blinded to any clinical attributes of the participants throughout the study.

Ethics, Assessment of Data, and Statistical Analysis

All patients provided written consent at screening, and the protocols were approved by local institutional research and ethical committees (CODEI and CEI). Anonymity was maintained using unique codes for each patient and sample. The stool gluten immunogenic peptide (GIP) concentrations determined by ELISA for individual samples were regarded as the gold standard. GIP concentrations below 0.156 μg/g were classified as negative (limit of detection), while concentrations above 0.64 μg/g were considered relevant, as this threshold is strongly associated with mucosal

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damage and immune activation [12]. The performance of the LFIA/PoCT was assessed by comparing its ability to detect samples with GIP concentrations at these levels as determined by ELISA. Analysis was conducted using Stata 16.1 (Stata Corp, College Station, TX). Sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, and agreement were determined by conventional formulas. These metrics were calculated for different stool GIP cut-offs (≥0.156 and ≥0.64 μg/g). Additionally, the agreement between the LFIA/PoCT tests and ELISA was quantified as a percentage and assessed using Cohen's kappa coefficient.

3. Results

Cohort Demography and Clinical Characteristics of Patients

Table 1 depicts demography and some clinical characteristics of the study population collecting samples for the study. Thirty-four percent of patients were symptomatic at entry the study, and 41.5% and 47.2% of them had IgA tTG and IgA DGP positive serology, respectively.

Table 1. Demography and some clinical characteristics of patients enrolled in the study.

Characteristics	
Number of patients/number of stool samples	53/299
Sex. F/M. (%)	48/5 (91/9)
Median age at entry the study (IQR). Yrs.	46 (34-55)
Median time on a gluten-free diet (IQR). Yrs.	8 (5-12)
Number of symptomatic patients (%). CSI score ≥38.	18 (34.0)
Celiac disease-related serology at enrolment	
IgA tTG antibodies. Number of positive cases	
>20 AU/mL (%).	22 (41.5)
IgA DGP antibodies. Number of positive cases	
>09 AU/mL (%).	25 (47.2)

Foot notes: F/M: female/male; IQR: Interquartile range; Yrs: years; CSI: celiac symptoms index; IgA tTG: Immunoglobulin A tissue transglutaminase; IgA DGP: Immunoglobulin A deamidated gliadin peptides.

Analysis of Stool GIP Determined by ELISA and Accuracy of Stool GIP Determinations by Using LFIA/PoCT

A total of 293 stool samples were collected. The performance of the LFIA/PoCT compared to the ELISA test in detecting GIP concentrations was determined at two different cutoffs. The findings suggest that the LFIA/PoCT demonstrates moderate sensitivity and high specificity when compared to the ELISA test, especially at the lower cutoff point of ≥0.156 μg/g. At this cutoff point, the LFIA/PoCT showed a sensitivity of 46.1% and a specificity of 88.8%. This indicates that while the LFIA/PoCT may miss detecting some positive samples identified by ELISA, it accurately identifies a high proportion of negative samples. The positive and negative predictive values, as well as the positive and negative likelihood ratios, further support the diagnostic performance of the LFIA/PoCT at this cutoff (Table 2).

Regarding the higher cutoff point of ≥0.64 μg/g considered, the LFIA/PoCT exhibited slightly higher sensitivity (55.4%) and maintained a high specificity (83.8%). The agreement between the LFIA/PoCT and ELISA tests, as indicated in Table 2, there was a substantial agreement between tests at this cutoff (78%) and but moderate as suggested by the Cohen's kappa coefficient. Overall, the study provides evidence supporting the utility of the LFIA/PoCT as a convenient and reliable option for detecting stool GIP concentrations, particularly in settings where access to laboratory-based ELISA testing may be limited. These findings could have important implications for improving and self-monitoring adherence monitoring in CeD patients.

Table 2. The statistical performance of the LFIA/PoCT for detecting stool GIP, compared with the quantitative determination of GIP concentration (in μg/g) by ELISA in the same stool samples.

 doi:10.20944/preprints202405.1632.v1

Stool GIP µg/g (ELISA)	N of patients (%)	LFIA/PoCT Sensitivity % (95% CI)	LFIA/PoCT Specificity % (95% CI)	LFIA/PoCT PPV % (95% CI)	LFIA/PoCT NPV % (95% CI)	LR+ % (95% CI)	LR- % (95% CI)	Agreement %	Cohen's *Kappa*
≥ 0.156	115/293 (39.5)	46.1 (36.8-55.6)	88.8 (83.2-93.0)	72.6 (60.9-82.4)	71.8 (65.4-77.7)	4.1 (2.6-6.5)	0.6 (0.5-0.7)	72	0.37
≥ 0.64	65/293 (22.2)	55.4 (42.5-67.7)	83.8 (78.3-88.3)	49.3 (37.4-61.3)	86.8 (81.6-91)	3.4 (2.4-4.9)	0.5 (0.4-0.7)	78	0.38

Foot notes: GIP: gluten immunogenic peptides; ELISA: enzyme-linked immunosorbent assay; N: number of patients; %: percentage; LFIA/PoCT: lateral flow immunoassay point-of-care test; PPV: positive predictive value; 95% CI: 95% confidence interval; LR+: positive likelihood ratio; LR-: negative likelihood ratio. 95% CI: 95% confidence interval.

4. Discussion

Periodic assessment of adherence to a GFD is essential for managing CeD effectively and preventing complications [1–3,11]. However, selecting the optimal tools and strategies for assessing adherence can be challenging. Our study aimed to address these challenges by utilizing stool GIP testing in real-life settings, providing a reliable indicator of gluten exposure [11]. We specifically focused on evaluating the effectiveness of a rapid qualitative home test, the LFIA/PoCT, in detecting stool GIP excretion. By comparing the LFIA/PoCT with quantitative ELISA testing, we aimed to determine whether the LFIA/PoCT could serve as a convenient and reliable option for monitoring GFD adherence.

Our findings contribute to the growing body of evidence supporting the use of stool GIP analysis for assessing adherence to the GFD. By demonstrating the effectiveness of the LFIA/PoCT in detecting stool GIP excretion, our study provides valuable insights into potential strategies for optimizing GFD monitoring in clinical practice. This study provides evidence that the LFIA/PoCT demonstrates a high ability to accurately detect negative stool GIP samples, as determined by quantitative ELISA, in a significant proportion of cases (88.8%). However, the performance of the LFIA/PoCT platform showed low sensitivities, adequate specificities, and predictive values for detecting stool GIP-positive samples according to the gold standard. These findings are consistent with previous observations made by our team and other research groups [4,5,7–9]. We hypothesize that the differences in performance between platforms could be attributed to different **sensitivities and to variations in the combinations of monoclonal antibodies and extraction** procedures utilized by each method [7]. Despite these limitations, the simplicity of the LFIA/PoCT for GIP detection and the high level of negative predictive value make it a convenient home-based method for self-assessment of dietary indiscretions. This suggests that while the LFIA/PoCT may not be as sensitive as ELISA for detecting GIP-positive samples, its ease of use and ability to reliably detect negative samples offer valuable benefits for individuals monitoring adherence to a gluten-free diet.

Our study possesses strengths that bolster the reliability and validity of the findings. Firstly, the substantial number of samples collected enhances the robustness of the analysis by minimizing potential variability that may arise from comparing different testing platforms. Secondly, the LFIA/PoCT was administered by experts in the field who were blinded to the ELISA testing results, ensuring unbiased interpretation and reducing the risk of subjective influence on results. Additionally, the utilization of stool GIP tests represents another strength, as stool is a biologically relevant material for assessing adherence to a GFD, as corroborated by recent research [9].

The real-life conditions of our study pose certain challenges in detecting significant amounts of stool GIP that may indicate immune activation and small intestinal damage. Given this consideration, we conducted an analysis of the performance of LFIA/PoCT for stool concentrations above 0.64 µg/g. The results revealed poor sensitivity and positive predictive value, but high specificity and negative predictive value for the qualitative test.

 doi:10.20944/preprints202405.1632.v1

5. Conclusions

Our study contributes to the ongoing efforts aimed at optimizing stool GIP testing for assessing adherence to the GFD, with potential implications for improving patient care and outcomes. We suggest that the repeated use of the LFIA/PoCT test by patients may enhance their understanding of adherence to the diet. Despite demonstrating lower sensitivity compared to the ELISA platform, the LFIA/PoCT test offers a simple and convenient method for patients to self-monitor adherence to the GFD. These insights empower patients to take an active role in assessing their adherence and have the potential to enhance overall patient care by enabling more targeted interventions and reducing unnecessary burdens on both patients and healthcare providers

Author contributions: JPS contributed to the study´s design, collected patients, statistical analysis of data and the writing and critical review of the manuscript. MPT performed the dietary assessment. ES, SN, MS collected patients and data acquisition. JCB contributed to the study´s design, analysis of data writing the manuscript. All authors approved the final manuscript.

Acknowledgments: GIP tests were generously provided by Biomedal S.L., Seville, Spain. No financial support was received.

Conflicts of interest: Authors have no conflicts of interest to declare.

Disclosures: JPS was speaker for Sanofi. JCB was speaker for Warfen and Biomedal SL., and research assessor for Takeda. Remaining authors have nothing to disclose.

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