PROTOCOL

Assessment of the adherence to a gluten-free diet in adolescent and adult patients with celiac disease: gluten immunogenic peptides management strategy

SOCIEDAD ESPAÑOLA DE ENFERMEDAD CELÍACA

AUTHORS

- **Marta Garzón Benavides**
- **Ángeles Pizarro Moreno**
  Servicio de Aparato Digestivo. Hospital Universitario Virgen del Rocío. Sevilla

COLLABORATORS

- **Ester Donat.** *Unidad de Gastroenterología y Hepatología Pediátrica. Hospital Universitario y Politécnico La Fe. Valencia*
- **Sergio Farrais.** *Servicio de Aparato Digestivo. Hospital Fundación Jiménez Díaz. Madrid*
- **Luis Fernández Salazar.** *Servicio de Aparato Digestivo. Hospital Clínico Universitario de Valladolid*
- **Marta Molero.** *Departamento de Gastroenterología y Elementos traza. Servicio de Análisis Clínicos. Hospital Universitario La Paz. Madrid*
- **Miguel Montoro.** *Unidad de Gastroenterología, Hepatología y Nutrición. Hospital Universitario San Jorge. Huesca*
- **Concepción Núñez.** *Laboratorio de investigación en Genética de enfermedades complejas. Instituto de Investigación Sanitaria del Hospital Clínico San Carlos. Madrid*
- **Carmen Ribes-Koninckx.** *Unidad de Enfermedad celiaca e Inmunopatología Diges-tiva. Instituto de Investigación Sanitaria La Fe, Hospital Universitario y Politécnico La Fe. Valencia*
- **Santos Santolaria.** *Unidad de Gastroenterología, Hepatología y Nutrición. Hospital Universitario San Jorge. Huesca*
- **Edurne Simón.** *Departamento de Farmacia y Ciencias de los Alimentos. Facultad de Farmacia. Universidad del País Vasco. Vitoria-Gasteiz*
- **Santiago Vivas.** *Servicio de Aparato Digestivo. Hospital Universitario de León*

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SCOPE OF APPLICATION

- Primary care
- Specialized care
- Multicenter
- Involves several Units or Services

ACTIVITY TO BE PROTOCOLIZED

- Diagnosis
- Treatment

PROFESSIONALS INVOLVED

- Family physicians
- Gastroenterologists
- Internal Medicine Specialists
- Biochemists
- Immunologists
- Dietitians-Nutritionists
- Patient associations

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1. DEFINITION OF THE PROBLEM

- Adherence to a strict and lifelong gluten-free diet (GFD) is currently the only available treatment for patients with celiac disease (CD) (Ludvigsson et al., 2014).

- The rigorous monitoring of GFD is challenging due to the ubiquity of gluten in foods, misinformation, variations in food labeling (Segura et al., 2021), and the presence of multiple factors leading to frequent cross-contact and gluten exposure (Muhammad et al., 2019).

- Non-adherence rates to the GFD of up to 69% and 64% have been described in adult and adolescent patients, respectively, based on adherence questionnaires, dietary records, serological tests, or the determination of immunogenic gluten peptides (GIP) in stool and urine (Segura et al., 2021). Furthermore, between 36% and 55% of patients do not achieve mucosal healing, despite considering their adherence to GFD to be correct. This has been linked to unintentional and unnoticed gluten exposures (Coto et al., 2021a).
- Proper adherence to the GFD allows symptom resolution and mucosal healing. However, lack of adherence carries a higher risk of adverse health effects such as anemia, osteoporosis, infertility, the onset of certain neoplasms (Marafini et al., 2020), and consequently, an increase in morbidity and mortality (Rubio-Tapia et al., 2013; Schiepatti et al., 2023).
- The diagnostic and management guidelines for CD from major scientific societies recommend periodic annual or biennial evaluation to assess adherence to GFD, symptom evolution, and potential complications. This evaluation should include clinical and nutritional assessment, dietary records, and comprehensive analytical testing with specific CD serology (Raiteri et al., 2022).

- The current monitoring scheme, with its existing tools, fails to ensure a high rate of adherence to GFD, resulting in persistent duodenal histological damage in a significant percentage of patients. Thus, over 50% of patients still exhibit atrophy two years after initiating GFD. Among them, 68% demonstrate adequate adherence according to dietary questionnaires, and over 70% are asymptomatic with negative CD serology. However, gluten exposure is detected through determination of GIP in stool in 77% of cases (Fernández-Bañares et al., 2021).
- Determining GIP in stool and urine allows for direct, accurate, and non-invasive assessment of gluten consumption (Comino et al., 2011, 2012, 2016, 2019; Moreno et al., 2016, 2017; Coto et al., 2021b, 2022).
- Regular, strict, and protocolized monitoring incorporating GIP determination will improve adherence and consequently increase the percentage of patients achieving mucosal healing (Garzón-Benavides et

al., 2023; Ruiz-Carnicer et al., 2020), as well as alleviating symptoms resulting from sustained gluten exposure or facilitating the determination of whether they might be due to other pathologies.

This protocol focuses on the management strategy of GIP in the follow-up of adolescent and adult patients with CD for monitoring adherence to GFD and decision-making based on its outcome.

2. TARGET POPULATION

- Adolescent patients with CD (over 14 years old) referred from pediatrics for transition to adult care.
- Adult patients (over 18 years old) with recently diagnosed well-documented CD or already on a GFD.

3. EXCLUSION CRITERIA

- Pediatric population.
- Patients on a GFD without a documented diagnosis of CD.

4. DEFINITION OF THE ACTIVITY TO BE CARRIED OUT

1. To review the available literature on adherence rates to GFD.
2. To update the current status of different GFD monitoring tools and outline their limitations in detecting gluten exposures.
3. To provide information about the advantages of determining GIP in stool and/or urine compared to other monitoring tools.
4. To develop a care protocol and a management algorithm for determining GIP in the follow-up of non-pediatric patients with CD to ensure proper adherence to the diet and avoid misdiagnosis in patients with persistent symptoms or villous atrophy.

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6. THEORETICAL OR CONCEPTUAL DOCUMENT

Introduction

Celiac disease (CD) is a systemic and chronic disorder primarily causing enteropathy of the small intestine[1]. It develops due to an inappropriate immune response to gluten peptides in genetically predisposed individuals. [1,2].

Currently, the only available treatment for CD is strict lifelong adherence to a gluten-free diet (GFD), which involves excluding gluten peptides from wheat, barley, rye, oats, and their hybrids such as triticale and derivatives from the diet[3]. Compliance with the GFD leads to symptom remission within days or months[4] and normalization of serological tests within the first 24-36 months[5]. However, mucosal healing in adult CD patients may require more time, as described in a study where intestinal villi recovery was present in only 34% and 66% of patients after 2 and 5 years of GFD initiation[6]. On the other hand, achieving proper adherence to GFD is challenging due to multiple factors such as the ubiquity of gluten in the food industry[7], lack of knowledge about gluten-containing foods, difficulty in interpreting product labels, high cost, or the need to not feel different in sociocultural events, all of which favor frequent gluten exposures[8]. Non-adherence rates to the GFD vary depending on the study population and the methodology employed. According to adherence questionnaires, serological tests, or determination of immunogenic gluten peptides (GIP) in stool and urine, variable percentages of dietary non-adherence have been described, ranging from 9-69% in adults and 14-64% in adolescents[7]. Considering age, adolescents constitute the group at highest risk of intentional non-adherence to the diet due to fear of social stigmatization[9-10], thus requiring rigorous monitoring during transition to adulthood to raise awareness of the disease and the responsibility of GFD adherence[9]. Furthermore, according to the results of intestinal biopsies from patients on GFD for at least 2 years, a lack of mucosal recovery has been

observed in 36-55% of the studied population[11–13]. Lanzini et al.[14] conducted a study with clinical, serological, and histological information from 463 adult CD patients, before and after starting GFD. Follow-up duodenal biopsy was performed after a median time on GFD of 16 months (range: 13-222 months), revealing that only a minority (8%) had normalized intestinal mucosa, and in 27%, histological lesions had not changed or had worsened[14]. Schiepatti et al. identified predictors of persistent villous atrophy including age over 45 at diagnosis, classical presentation (chronic diarrhea with symptoms and signs of malabsorption), lack of clinical response to GFD, and poor diet adherence. Lack of adherence to GFD was the most important predictor (OR 49.3 95% CI 26.3-92.2)[15]. Similarly, most studies consider food contamination and inadvertent gluten exposure to play a crucial role in this lack of progression towards mucosal healing[14,16–18]. It is estimated that the average gluten exposure for many patients may exceed 100 mg/day, with some individuals experiencing over 600 mg/day, amounts sufficient to result in persistent symptoms, villous atrophy, and long-term complications[19,20].

The persistence of villous atrophy and inflammation is associated with increased morbidity and mortality, with significantly shorter complication-free and survival times compared to patients who achieve mucosal recovery. It is also the main risk factor for developing complications (HR 9,5 IC 95% 4,77-19,4)[15], such as fractures related to osteoporosis, anemia and other nutritional deficiencies, infertility, and intestinal neoplasms, particularly intestinal lymphoma[1,5,6,21]. Compared to the general population, patients with CD and persistent villous atrophy have a 3.78 (IC 2,71-5,12) times higher risk of intestinal lymphoma, compared to 1.5 (IC 0,77-2,62) times for those with mucosal recovery[5,22].

In summary, the benefits of adherence to GFD in reducing gluten-induced inflammation and its effects on various organs result in improved quality of life and a lower risk of complications[5]. Therefore, strict monitoring of GFD adherence is advisable.

This document reviews the current status of different procedures for monitoring GFD adherence and establishes an algorithm for using GIP in the follow-up of adolescent or adult CD patients.

Monitoring adherence to a gluten-free diet

Adherence to GFD is reinforced with the regular follow-up of the CD patient[1,23]. It is established that the monitoring frequency should be every 3-6 months in newly diagnosed patients, and subsequently annually or biennially, indefinitely, once the patient is stable,

symptom-free, with serological normalization, and adequately adhering to GFD[1,5,24]. However, this monitoring scheme does not prevent a high percentage of patients from being improperly adhered to GFD, as mentioned earlier. Garzón-Benavides et al.[23] conducted strict monitoring with four reviews over a year in 94 patients on GFD for at least 24 months. At each visit, clinical assessment, serological determination, adherence questionnaire, and determination of GIP in urine were performed, observing a significant reduction in the percentage of patients with GIP detection and persistent villous atrophy at the end of follow-up. Furthermore, a relationship between the frequency of GIP detection and histological lesion was demonstrated.

These results led to the suggestion that more frequent interval follow-ups, instead of the annual follow-up typically conducted in clinical practice, improved adherence to GFD, with subsequent histological improvement[23]. Therefore, regular follow-up at shorter intervals could achieve greater adherence to GFD. Key aspects of periodic medical follow-up include assessing symptom resolution, mucosal healing, potential complications, improvement in quality of life, and monitoring compliance and adherence to the GFD[5].

The tools currently available to ensure adherence to the GFD include clinical evaluation, nutritional status assessment, CD serology, adherence questionnaires and dietary records, duodenal biopsy, and determination of GIP [1,5,24].

Clinical and Nutritional Assessment

As previously mentioned, one of the goals of the GFD is symptom resolution and improvement of intestinal absorption. However, the GFD itself has limitations in providing certain nutrients, making regular clinical and nutritional assessment crucial in the follow-up of these patients. Nevertheless, clinical assessment should not be correlated with mucosal recovery given the limited value of clinical symptoms as predictors of villous atrophy[25], which can persist even in the absence of symptoms[26–28]. The correlation between clinical symptoms and the severity of histological lesions is very poor in adult patients at the time of diagnosis, and therefore, this correlation is not expected to improve during follow-up when the patient is on GFD[29,30]. Thus, clinical response cannot be used as an indicator of diet adherence and mucosal recovery in asymptomatic or paucisymptomatic patients at diagnosis[31]. On the other hand, over 70% of patients with persistent villous atrophy two years after starting GFD are asymptomatic[28], demonstrating the limited value of clinical evaluation in assessing mucosal healing. Additionally, throughout follow-up, patients may experience gastrointestinal symptoms similar to those of CD. It is necessary to determine whether these symptoms are due to recurrent gluten exposures secondary to poor adherence to GFD, other associated entities, or functional mechanisms that may be partly motivated by changes in fiber intake associated with the GFD[32].

TABLE 1. Key aspects in clinical assessment, nutritional evaluation, and complication development.

CLINICAL ASSESSMENT	NUTRITIONAL EVALUATION	COMPLICATION DEVELOPMENT
Dyspepsia	Weight. Height, and BMI	Osteopenia / Osteoporosis
Flatulence	Complete blood count General biochemistry Coagulation Iron metabolism Thyroid hormones Calcium, phosphorus, magnesium, folate, cobalamin (B12) Copper, selenium, zinc, and vitamins A, D, E, K, B complex	Infertility
Diarrhea		Autoimmune hepatitis
Abdominal pain		Lymphoproliferative disorders of the SI / Other neoplasms
Fatigue		RCD

Abbreviations: *BMI, body mass index; SI, small intestine; RCD, refractory celiac disease.*

Therefore, it is essential to perform regular and indefinite clinical and nutritional evaluation of the patient with the aim of improving the patient's quality of life and assessing the development of complications **(Table 1)**. However, this assessment has limited utility as a monitoring tool for adherence to GFD. **Table 1** shows the key aspects in the clinical assessment, nutritional evaluation, and screening for complications in patients with CD.

Celiac Disease Serology

Serology is a frequently used marker of adherence in monitoring GFD. It is well known that CD antibodies are highly valuable in disease diagnosis due to their high diagnostic accuracy, with a specificity, positive predictive value (PPV), negative predictive value (NPV) of tissue transglutaminase type 2 (anti-TG2) IgA and endomysial antibodies (EMA) of approximately 98%, 72%, 99%, and 99%, 83%, and 99%, respectively[33,34]. However, they are poor predictors of dietary transgressions[20,35,36] and have low sensitivity for detecting villous atrophy during follow-up (50% and 45% for anti-TG2 and EMA, respectively)[37]. All of them are gluten-dependent, so there will be a decrease in their baseline levels until normalization around 24-36 months after starting GFD[14]. There are numerous studies demonstrating that serology once negative does not become positive again in a large portion of patients who commit transgressions[20,35–37]. In fact, more than 80% of patients who maintain villous atrophy after more than 2 years on GFD have negative anti-TG2 antibodies[23].

Therefore, the utility of serology in monitoring is limited to the first months after starting the GFD, so that their decrease until normalization indicates a reduction in gluten consumption. However, once normalized, they are not capable of detecting gluten exposures in low quantities[38] or identifying the persistence or recurrence of villous atrophy[23]. Consequently, the value of serology in long-term follow-up in these patients is very limited.

Structured questionnaires and dietary records

The review of GFD supported by questionnaires that assess adherence and frequency of consumption of certain foods reported by the patient themselves is a tool to detect gluten consumption and, through them, promote education towards a proper diet[1,24]. There are different adherence questionnaires, such as the one by Biagi or the *Celiac Dietary Adherence Test* (CDAT) by Leffler, with the latter being the only one translated and validated in Spanish. It allows for a quick evaluation with 7 questions that assess: CD symptoms, self-efficacy expectation, reasons for maintaining a GFD, knowledge of the condition, associated risk behaviors, and perceived adherence level[39,40]. However, these structured questionnaires are subjective and cannot identify involuntary infractions that the patient cannot detect, as it has been described that 30% of patients consume gluten unintentionally and 20% cannot identify the transgression[41]. Furthermore, they have low sensitivity in detecting villous atrophy, with sensitivities of 55% and 25-33% for the CDAT and Biagi questionnaires, respectively, so their applicability in clinical practice is limited[5].

Evaluation by an expert dietitian in CD is highly valuable to identify limitations in dietary knowledge or practices associated with a high risk of inadvertent gluten exposure. Currently, there is no standardized tool that allows a nutritionist to objectively assess GFD compliance.[42]. Dietary records have been developed, such as the standardized dietary interview (SDI)[43] and the DIET-GFD related to the risk of gluten exposure and estimated consumption[42]. However, their external validity has not been demonstrated[10,42]. The lack of objective tools makes it necessary to accurately collect in the patient's medical history their dietary habits (food preparation method, ingredients of prepared dishes, containers used, brands of commercial products, restaurants, food stores), and other issues related to cross-contact, to know if the patient identifies and avoids sources of gluten exposure[10].

On the other hand, the expert dietitian in CD plays a fundamental role in promoting healthy eating, expanding options for alternative nutritious foods, and discouraging unnecessary restrictive dietary practices[1,3,5]. In this way, deficiencies of micro and macronutrients that may occur during treatment can be avoided, as well as constipation, which is frequent

in these patients due to the low fiber content of GFD, requiring supplementation with other fiber-rich foods[5].

Very few healthcare units have specialized nutritionists or dietitians, which constitutes a significant barrier to proper diet teaching and guidance in these patients

Intestinal biopsy

One of the objectives of adhering to a GFD is mucosal healing, which is the main marker of response to GFD. Its assessment requires an oral endoscopy and the taking of biopsies from the duodenum. Although it is a technique with few risks and tolerance has improved thanks to deep sedation with propofol, it remains an invasive procedure[44]. In fact, it is not included in clinical guidelines, and there is not enough data and evidence to support the need for regular endoscopic monitoring in long-term follow-up[5].

Guidelines from major scientific societies only recommend performing an intestinal biopsy 1-2 years after starting GFD to verify mucosal recovery, especially important in those patients at higher risk of persistent villous atrophy, such as those diagnosed at advanced ages (over 40 years) or those presenting severe villous atrophy at the time of diagnosis[1,5,45]. The problem is that, in many cases, one year is too short a time interval to achieve mucosal recovery[46].

The persistence of duodenal histological lesions after one year of follow-up can lead to frustration in those patients who adhere to GFD correctly and cause excessive concern about gluten exposure, leading to anxiety and depression[47]. Therefore, a more advisable option may be to perform an endoscopy 2 years after starting the GFD. Its performance should always be individualized in those patients with persistent symptoms or nutritional deficits at any time during the treatment, and in those where the persistence of duodenal histological lesions needs to be evaluated[5]. Given the importance of the histological result in the correct interpretation of adherence and response to GFD, it is essential to improve the quality of duodenal biopsies, avoiding poor representativeness. Therefore, a total of 4-6 biopsies should be taken one by one (including 2 from the bulb), attempting a correct orientation of the samples and avoiding shaking the forceps inside the container.[48].

Similarly, a correct histological assessment by experts in digestive pathology is essential, where the report includes standardized information on the suitability of the sample, the increase in intraepithelial lymphocytes, the crypt:villus ratio, and the degree of atrophy[49,50].

As mentioned previously, histological evaluation should be considered not only at 2 years after starting GFD but also when there is no adequate clinical response to it. However, it is well known that persistent villous atrophy often occurs in asymptomatic patients[28]. Therefore,

it is essential to identify patients at high risk of histological lesions using non-invasive tools.

Gluten Immunogenic Peptides in Stool and Urine

Determining GIP in human samples (stool and urine) is considered a useful tool in monitoring adherence to GFD[1,17,24]. GIPs are fragments of gluten resistant to gastrointestinal digestion and are the main triggers of the immune response in celiac patients[51,52]. The recovery of measurable amounts of GIP in feces or urine directly indicates that gluten has passed through the digestive tract, demonstrating voluntary or involuntary gluten consumption non-invasively with high sensitivity and specificity[13,19,20,35,53]. GIPs are eliminated in the feces, although some may pass through the basolateral membrane of enterocytes, enter the portal circulation, reach the kidneys, and after a process of ultrafiltration, be partially or totally excreted in urine[54,55]. Determining these peptides in feces is done through ELISA and lateral flow immunoassay (LFIA) techniques, and in urine through LFIA, allowing direct and non-invasive assessment of gluten consumption[13,53,56,57]. Despite some individual variability, the time between gluten consumption and the onset of GIP detection in feces varies between 1 and 3 days, with a maximum detection time of 7 days. In urine, the first 3-9 hours after ingestion have the highest GIP concentration, and although the probability of detection decreases afterward, their presence has been described in some cases up to 36 hours post-ingestion[58].

Multiple studies with diverse methodology have compared the determination of GIP in feces and urine with clinical manifestations, adherence questionnaires, and serological tests, demonstrating the greater ability of GIP to detect gluten exposure in the diet[20,35,59–62]. For example, Fernández-Bañares et al.[28] followed 72 adult patients with *de novo* CD for 2 years using clinical assessment, serology, adherence questionnaires, and determination of GIP in feces. They observed that 68.4% of patients showed good or excellent adherence to GFD according to questionnaires. However, 53% of all patients still had villous atrophy 2 years after starting GFD, with 72.5% of them being asymptomatic and 75% having negative serology. However, GIPs were detected in at least one stool sample in 77% of patients with persistent villous atrophy[28]. Similarly, Ruiz-Carnicer et al.[27] analyzed the clinical utility of determining GIP in urine for monitoring adherence to GFD and its usefulness as a predictor of duodenal histological lesions by correlating the punctual determination of GIP with the degree of duodenal histological lesions. They demonstrated that measuring GIP in 3 urine samples over a period of 7 days, including the weekend, was the best option to confirm adherence to GFD due to the high sensitivity (94.4%) and negative predictive value (96.8%) obtained in relation to duodenal biopsy findings[27].

Later, in the work of Garzón-Benavides et al.[23] the relationship

between serial determination of GIP in urine (6 samples over a year) and histological evolution is evidenced. Thus, in those patients with histological normality or mucosal recovery, the detection of GIP in urine decreases over follow-up. In contrast, in those with persistent villous atrophy, the percentage of patients with GIP detection is higher and does not change over follow-up. This demonstrates that frequent detections of GIP, even in small concentrations, often have histological repercussions. There is also a relationship between the number of urines with GIP detection (more than 4 urines with GIP detection over a year) and the presence of histological lesions, and similarly, the repeated absence of GIP in 2 or more visits throughout a year correlates with the absence of histological lesions[23]. According to these results, serial determination of GIP in the long-term follow-up of patients with CD provides guidance on adherence to GFD and the degree of duodenal histological lesion. Thus, the best strategy for monitoring adherence to GFD appears to be the semiannual determination of GIP in feces or urine.

7. OPERATIONAL DOCUMENTS

Protocol for the management of gluten immunogenic peptides in the follow-up of celiac disease

Initially, a series of considerations are described to optimize sample collection:

1. Stool samples

- It is recommended to collect two separate stool samples on 2-3 days across the week prior to the medical review, including one weekday and one reflecting weekend intake. Thus, there are different possible collection schedules: preferably, Monday-Thursday or Tuesday-Saturday, and if not possible due to the patient's lifestyle or eating habits, another option would be, for example, Wednesday-Sunday.
- Collecting one of the samples reflecting weekend intake is recommended because gluten exposure is more likely when eating out. This schedule can be adapted according to the patient's lifestyle.
- It is recommended to store the samples in a freezer at -20 °C until transportation to the hospital, and subsequently keep the samples frozen at -20 °C for up to 24 months.
- It will be considered that the patient is not exposed to gluten in the assessment of one week if GIP is not detected in any of the two stool samples. On the contrary, they will be exposed to gluten if GIP is detected in at least one of the two stool samples.

2. Urine samples

- It is recommended to collect 3 urine samples throughout the week prior to the medical review, with 2 samples during the weekdays and one reflecting weekend intake, with the same proposed objective as in stool

samples. Therefore, there are several possible urine collection schemes, but at least one weekend sample should be included. Thus, a possible scheme could be: Monday-Wednesday-Saturday night or Sunday morning. This schedule can be adapted according to the patient's lifestyle.

- In choosing the optimal time for urine collection, collecting the first morning urine is recommended as it is more concentrated, given the inverse relationship between GIP detection and the amount of liquid ingested. However, another option would be after dinner, reducing or avoiding liquid intake as much as possible for the 6 hours prior to urine collection, as the period with the highest percentage of GIP detection is 6-9 hours post-ingestion.
- It is recommended to store the samples in the freezer at -20 °C until transportation to the hospital, and subsequently keep the samples frozen at -20 °C for up to 12 months.
- It will be considered that the patient is not exposed to gluten in the one-week assessment if GIP is not detected in any of the 3 urine samples, and they will be considered exposed to gluten if detected in at least one of the 3 samples.

Below is the scheme for collecting GIP in stool and urine samples in the diagnosis and long-term follow-up of patients with CD **(Fig. 1)**. Performing a GIP determination at the time of diagnosis will help identify those patients who reduce gluten consumption prior to duodenal biopsy, allowing for correct interpretation of histological results. For this purpose, it is recommended to collect a single urine (morning) or stool sample on the same day or the day before duodenal biopsy. In individuals newly diagnosed with CD who initiate a GFD, a first review could be conducted between 3 and 6 months after diagnosis, given the difficulty in adaptation and learning involved in the GFD during the first year after CD diagnosis. Performing both GIP and serology at the beginning of follow-up helps guide patients and specialists on adherence to GFD. Thus, a decrease in antibody levels accompanied by the persistence of GIP in most stool or urine samples indicates a decrease in gluten intake, but does not ensure correct adherence, so reinforcement and clarification of any doubts the patient may have about proper implementation are necessary. Conversely, if GIP determinations in stool or urine are negative, the patient has adhered correctly to the diet, and the decrease in antibody levels is within its natural evolution toward normalization, alleviating the possible anxiety and stress of the patient by not having antibodies negativized.

In patients with well-documented CD who are on a GFD, semiannual determinations of GIP should be performed. In asymptomatic patients, with normalization of CD serology, mucosal recovery, and absence of GIP in successive medical reviews over 24 months, annual follow-up could be

FIGURE 1. Strategy for determining gluten immunogenic peptides in the diagnosis and follow-up of patients with celiac disease.

considered, shortening the interval of GIP determination if there is a change in the patient's clinical status before the annual review.

Interpretation of Long-Term Results

- Absence of GIP in successive visits (no detection of GIP in any of the samples throughout the year) will suggest the patient's appropriate adherence to the diet.
- Detection of GIP in any of the collected samples indicates that the patient has been exposed to gluten. The frequency of GIP detection during follow-up will reveal how often the patient is exposed to gluten and the likelihood of presenting duodenal histological damage. It has been described that the presence of more than 4 urine samples throughout 1 year with GIP detection predicts histological damage with a specificity of 93%. In summary, GIP detection indicates the need to reinforce adherence to the GFD, preferably in specialized dietary consultations.
- In case of no clinical response to the GFD, serial determination of GIP will help determine whether it is due to poor adherence to the diet or, conversely, if adherence is correct (as evidenced by the

repeated absence of GIP in successive visits). In this case, it should be assessed whether the symptoms are due to the coexistence of other clinical entities **(Table 2)**.

- If after the relevant tests, no associated pathology is found to justify the persistence of symptoms or abnormal laboratory results, a new duodenal biopsy is recommended:

  - Histological normality and repeated absence of GIP would suggest a functional origin of the symptoms.

  - Persistence of villous atrophy needs evaluation of whether the diagnosis of CD was correct **(Table 3)**, if there are other causes of villous atrophy **(Table 4)** or the development of refractory celiac disease (RCD).

Figure 2 outlines the follow-up algorithm for CD including the use of GIP.

TABLE 2. Clinical conditions associated with celiac disease that could explain the persistence of symptoms despite a gluten-free diet.

CLINICAL ENTITY	DIAGNOSTIC TEST
Sugar intolerance - Lactose - Fructose - Sorbitol	Hydrogen Breath Test
Small Intestinal Bacterial Overgrowth	Hydrogen Breath Test (Glucose, Lactulose)
Microscopic Colitis	Sequential Colon Biopsies
Exocrine Pancreatic Insufficiency	Test with ^{13}C-labeled triglycerides Fecal elastase
Crohn's Disease	Inflammation biomarkers: PCR, ESR, FCP MR Enterography Enteroscopy
Bile Salt Malabsorption	Gammagraphy for bile acid malabsorption (SeHCAT) Resincolestiramine therapeutic trial: 8 g for 10 days
Irritable Bowel Syndrome	Rome IV Criteria Exclusion of other pathologies

Abbreviations: *^{13}C, Carbon 13; PCR, C-reactive protein, ESR, erythrocyte sedimentation rate, FCP, fecal calprotectin; MRI, magnetic resonance imaging; SSeHCAT, 75-selenium homocholic acid taurine gammagraphy (adapted from Prodiggest Project: Evaluación diagnóstica del paciente con sospecha clínica de enfermedad celiaca y atrofia vellositaria seronegativa, 2020).*

TABLE 3. Criteria for assessing a correct diagnosis of celiac disease.

Presence of compatible symptoms
Positivity of anti-TG2 or EMA antibodies at any point in the course
Histological findings consistent with celiac disease
Skin biopsy compatible with dermatitis herpetiformis
Presence of HLA DQ2 (DQ2.5 and/or DQ2.2) and/or DQ8
First-degree relatives affected by celiac disease
Concomitant autoimmune diseases

Abbreviations: *Anti-TG2, anti-tissue transglutaminase type 2; EMA, antiendomysial antibodies; HLA, human leukocyte antigen (modified from 24).*

8. REQUIRED RESOURCES

This protocol aims to guide clinical care and diagnostic and therapeutic decision-making, based on the determination of GIP, in monitoring adherence to the GFD in patients with CD. The minimum resources needed for protocol implementation are specified below.

- Testing Site
- Personnel
- Clinical-diagnostic Material
- Economic resources

Testing site

This protocol is applicable in the primary care setting with physicians specialized in CD, as well as in general gastroenterology clinics or specialized clinics focused on managing patients with CD and other gluten-related disorders. These services can be provided in both public and private healthcare facilities.

Personnel

Centers adhering to the protocol should have gastroenterologists, biochemists, immunologists, pathologists specialized in digestive pathology, and preferably, dietitians or nutritionists specialized in CD.

Clinical-diagnostic materials

The implementation of the protocol requires:

1. Laboratory materials for basic studies including:

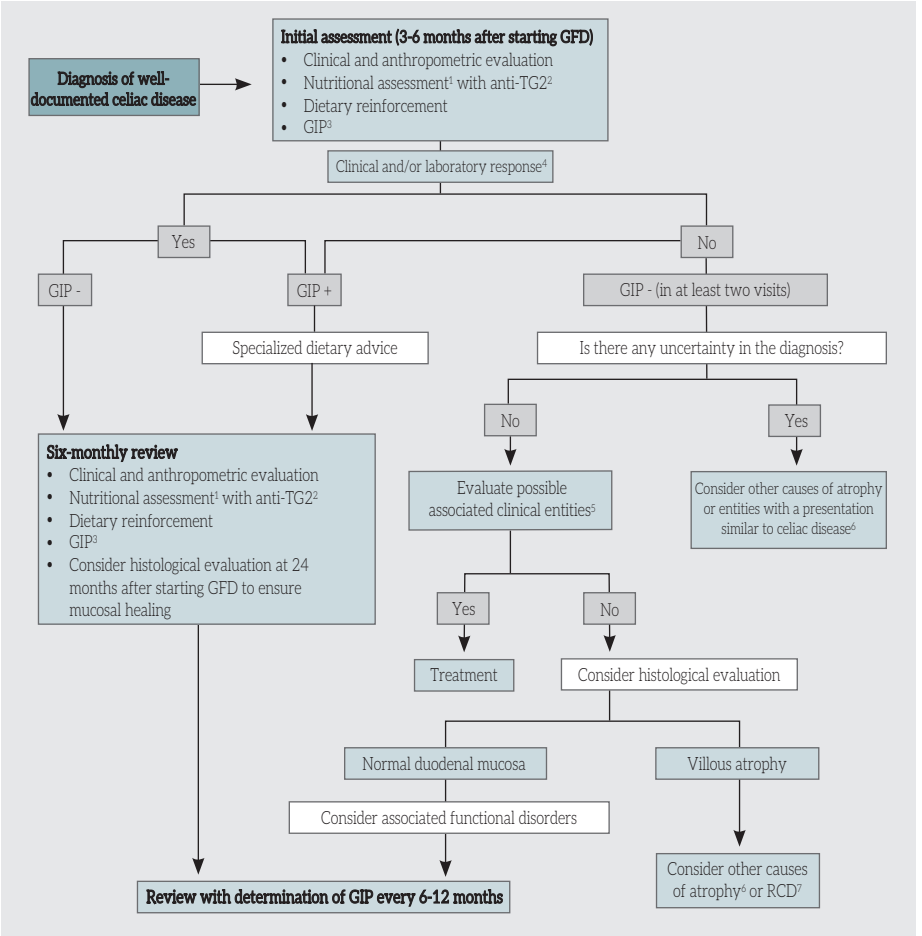
   a. Complete blood count
   b. Biochemistry
   c. Immunology
   d. Genetics
   e. Microbiology

TABLE 4. Main causes of non-celiac villous atrophy and features aiding in the differential diagnosis.

CLINICAL-PATHOLOGICAL ENTITY	CLINICAL-PATHOLOGICAL FEATURES AIDING IN DIFFERENTIAL DIAGNOSIS
Immune-mediated	
Crohn's Disease	Typical endoscopic appearance. Histological presence of transmural inflammation, non-caseating granulomas, fibrous tracts, and alteration of crypt architecture
Food Allergies	Relationship between symptoms and specific foods. Positive serological IgE tests or skin tests. Predominance of eosinophils in histology
Eosinophilic Enteritis	Dense infiltration of eosinophils in the small intestine
Autoimmune Enteritis	History of other autoimmune diseases. Presence of anti-goblet cell and anti-enterocyte antibodies. Heterogeneous pattern of lymphocytic infiltration of the small intestine
Graft-versus-host disease	History of organ transplantation
Common variable immunodeficiency	Low levels of immunoglobulins. Respiratory and other organ infections. Absence of plasma cells in lamina propria
Microbial	
Tropical sprue	Travel to endemic areas (Caribbean, South India, Southeast Asia)
Tropheryma whipplei (Tw)	PAS-positive macrophages. Demonstration of Tw DNA by PCR
Mycobacterium tuberculosis	Granulomas in the mucosa. Positive Quantiferon test
Medication	
NSAIDs	History of NSAID consumption
Olmesartan, candesartan	History of hypertension with the use of these drugs
Neoplasms	
Immunoproliferative Small Intestinal Disease (IPSID)	Dense infiltration of plasma cells in lamina propria. Presence of aberrant lymphocytes in lymphoma study
Lymphoma	Histological lesions compatible with lymphoma on histological examination
Metabolic and Degenerative	
Abetalipoproteinemia	Limited mainly to childhood. Histological demonstration of intracytoplasmic vacuoles.
Lymphangiectasia	Slightly dilated intestinal villi. Acellular mass, displacement along lymphatic vessels
Amyloidosis	Amyloid deposits in the mucosa (stained with Congo red)
Mastocytosis	Infiltration of mast cells (toluidine blue staining)
Others	
Collagenous sprue	Mucosal atrophy and excessive collagen deposition at the subepithelial level

Abbreviations: *PAS, Periodic Acid-Schiff staining; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; NSAID, nonsteroidal anti-inflammatory drug; HTA, arterial hypertension (modified from Prodiggest Project: Evaluación diagnóstica del paciente con sospecha clínica de enfermedad celiaca y atrofia vellositaria seronegativa, 2020).*

FIGURA 2. Algorithm for the follow-up of celiac disease.



[1]Complete blood count, comprehensive metabolic panel, thyroid hormone, coagulation profile, iron metabolism, calcium, phosphorus, magnesium, vitamin D3, folate, and cobalamin (B12). In the presence of a classical presentation pattern (malabsorptive diarrhea and weight loss) or in cases of severe watery diarrhea, consider determining levels of copper, selenium, zinc, and vitamins: A, E, K, riboflavin (B2), niacin (B3), pyridoxine (B6), biotin (B7). Consider periodic determination of these micronutrients in the patient's follow-up when there are doubts about the completeness and balance of the gluten-free diet (GFD). [2]Determination of anti-TG2 antibodies until negativization. [3]Collection of 3 urine samples or 2 stool samples according to the proposed scheme. At each review, if one of the samples is positive, refer the patient to specialized dietary consultation. If all samples are negative for GIP determination, continue with the established review scheme. [4]Decision pathway applicable to each clinical review in the long-term follow-up of the patient, from diagnosis. [5]Small intestinal bacterial overgrowth, microscopic colitis, exocrine pancreatic insufficiency, lactose/fructose/sorbitol intolerance, inflammatory bowel disease, bile acid malabsorption, irritable bowel syndrome. [6]Autoimmune enteropathy, common variable immunodeficiency, Crohn's disease, eosinophilic gastroenteritis, mastocytosis, drugs (e.g., olmesartan), parasitic infections (e.g., giardiasis), other infections (e.g., tuberculosis), Whipple's disease, abetalipoproteinemia. [7]Consider in cases of persistent malabsorption symptoms and villous atrophy at 12 months after starting the GFD.
***Abbreviations:** CD, celiac disease; GFD, gluten-free diet; anti-TG2, anti-tissue transglutaminase 2 antibodies; GIP, gluten immunogenic peptides; RCD, refractory celiac disease.*

2. Laboratory Material for GIP Analysis in Stool or Urine. Rapid detection kits for GIP in stool or urine using LFIA are required, as well as standard laboratory materials such as urine collection bottles, pipettes (100 µL – 1000 µL), disposable tips, plastic Eppendorf® tubes, 96-100% ethanol (for stool samples only), and powder-free gloves. If the ELISA technique is used for stool analysis, an ELISA kit optimized for GIP detection along with the standard laboratory materials mentioned above will be required. Additionally, a plate reader with a 450 nm filter, a vortex mixer, a thermostatic bath adjustable to 50 °C are required, whilst it is also advisable to have a multichannel pipette and an automatic plate washer.

3. Gastrointestinal Endoscopy Unit equipped with the necessary equipment for performing upper gastrointestinal endoscopy and other procedures as necessary (colonoscopy, enteroscopy, and capsule endoscopy).

4. Pathology Laboratory with pathologists specialized in digestive pathology who can accurately identify and define histological lesions associated with celiac disease.

5. Facilitate access to consultations with Dietitians-Nutritionists specialized in celiac disease for identifying possible sources of gluten exposure and making appropriate modifications to the gluten-free diet.
6. Resources required for identifying other conditions:
   a. Functional gastrointestinal laboratory for conducting:
      i. Hydrogen breath tests for sugar intolerance (lactose, fructose, sorbitol)
      ii. Small intestinal bacterial overgrowth.
   b. Fecal elastase or ^{13}C-triglyceride breath test for studying exocrine pancreatic insufficiency.
   c. Radiodiagnostic Service for performing Abdominal Computed Tomography or Magnetic Resonance Imaging (MRI), including enterography.

Economic Resources

The clinical, diagnostic, and therapeutic management for monitoring adherence to GFD according to the presented protocol requires human, laboratory, and radiology resources already included in the service portfolio of hospitals and clinics.

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