Revista Española de Enfermedades Digestivas
The Spanish Journal of Gastroenterology

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DOI: 10.17235/reed.2025.11519/2025
Link: PubMed (Epub ahead of print)

Please cite this article as:
Pizarro Moreno Ángeles , Garzón Benavides Marta, Comino Isabel, Donat Esther, Farrais Villalba Sergio, Fernández Salazar Luis, Molero Marta, Montoro Huguet Miguel, Ribes-Koninckx Carmen, Santolaria Santos, Simón Edurne, Sousa Carolina, Vivas Santiago , Núñez Concepción . Evaluation of adherence to the gluten-free diet in adolescent and adult patients with coeliac disease: a strategy based on the determination of gluten immunogenic peptides endorsed by the Spanish Society of Coeliac Disease (SEEC). Rev Esp Enferm Dig 2025. doi: 10.17235/reed.2025.11519/2025.

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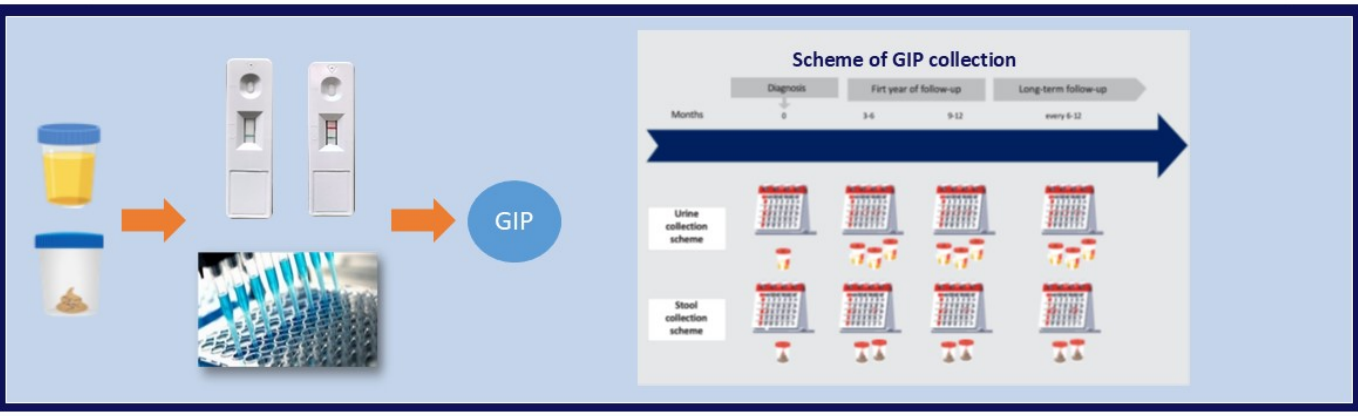
EVALUATION OF ADHERENCE TO THE GLUTEN-FREE DIET IN ADOLESCENT AND ADULT PATIENTS WITH COELIAC DISEASE: A STRATEGY BASED ON THE DETERMINATION OF GLUTEN IMMUNOGENIC PEPTIDES ENDORSED BY THE SPANISH SOCIETY OF COELIAC DISEASE (SEEC)



Adherence to the gluten-free diet remains a major challenge in celiac disease, with over 60% of patients showing unintentional gluten exposure. Traditional follow-up methods, including serology, often fail to detect transgressions. The determination of gluten immunogenic peptides (GIP) in stool or urine provides an objective, non-invasive tool to monitor adherence. A protocolized follow-up based on GIP testing improves dietary compliance and promotes mucosal healing.

Pizarro-Moreno, et al. 2025

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Evaluation of adherence to the gluten-free diet in adolescent and adult patients with coeliac disease: a strategy based on the determination of gluten immunogenic peptides endorsed by the Spanish Society of Coeliac Disease (SEEC)

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Conflict-of-interest statement: Isabel Comino and Carolina Sousa are inventors of the patent "Determination of levels of gluten immunogenic peptides in human samples" (No. WO/2012/089868), and Carolina Sousa is inventor of the patent “Detecting gluten peptides in human fluids" (No. WO/2016/005643).
All other authors report no conflicts of interest.

Financial resources: The clinical diagnostic and therapeutic management of adherence monitoring to the GFD according to the protocol presented here requires the human, laboratory and radiodiagnostic resources already incorporated in the service portfolio of hospitals and clinics.

Ethical considerations: This study did not require informed consent, as no personal or identifiable data were collected from participants.

Abstract

Adherence to a gluten-free diet (GFD) is the only treatment for patients with celiac disease (CD) with non-compliance rates higher than 60% and 55% of patients with persistent atrophy related to inadvertent gluten exposure.
The main scientific societies recommend a periodic annual or biennial evaluation with nutritional assessment, dietary record and specific laboratory and serological tests to assess adherence to GFD, symptoms and complications.
Most patients on GFD are asymptomatic with negative serology, but the determination of gluten immunogenic peptides (GIP) in stool is positive in 77% of them.
A protocolized follow-up with GIP determination will improve adherence and mucosal healing. This protocol focuses on the GIP management strategy in the follow-up of CD patients to monitor adherence to DSG and make decisions based on the results.

Keywords: Coeliac disease. Gluten-free diet. Adherence. Monitoring. Gluten immunogenic peptides.

Objectives and methodology for the writing of the protocol

The consensus document, developed by the Spanish Society of Coeliac Disease (SEEC), employed a rigorous methodological process to ensure the validity of its clinical recommendations. Initially, a working group drafted a first version based on available scientific evidence and clinical experience. Subsequently, a peer review process was implemented to incorporate a multidisciplinary perspective and ensure the quality of the recommendations. Experts with recognised experience and knowledge in the specific thematic area, all members of the society and other national and international societies, were invited to participate. Anonymity was maintained in both directions to encourage critical and objective evaluation. Reviewers exhaustively evaluated the content, clarity, coherence, and applicability of the recommendations, providing constructive comments and suggestions. All observations received were carefully analysed and discussed by the working group, resulting in the review and refinement of the final document.

All authors were invited because of their experience, prestige, academic recognition and representation in their respective societies. Some of the authors worked on the initial reference document, which was reviewed and corrected by the whole group and then presented at the SEEC congress. Subsequently, it was disseminated among SEEC members and submitted for internal and external peer review through the standard procedures of Clinical Gastroenterology and Hepatology.

The main objective of the project was to develop a healthcare protocol for the management of gluten immunogenic peptides (GIP) determination in faeces and urine in the follow-up of patients with coeliac disease as a tool for monitoring adherence to the gluten-free diet (GFD). Therefore, we reviewed the literature on current GFD monitoring tools and their ability to detect cross-contacts or dietary transgressions throughout the follow-up as well as all available literature on GIP detection, their sensitivity and specificity in detecting gluten peptides in body fluids and their possible application to clinical practice.

The protocol aimed to provide guidance on how to use GIP determination in the follow-up of the coeliac patient with the intention of improving follow-up by avoiding under-diagnosis of persistent villous atrophy and clarifying the origin of persistent symptoms in some patients and assessing the possible diagnosis of refractory coeliac disease.

This protocol is relevant for physicians and nurses treating patients with CD and is intended for use in adolescent and adult patients with this condition, in both primary care and hospital care settings.

The current monitoring of CD is an interdisciplinary approach, which may involve general practitioners, gastroenterologists, paediatricians, endoscopists, nurses and clinical nutrition experts or nutritionists who will hopefully see their work simplified and oriented with the application in monitoring the use of GIP determination following the present protocol for use.

THEORETICAL OR CONCEPTUAL DOCUMENT

Introduction

Coeliac disease (CD) is a chronic, systemic disorder that results primarily in small intestinal enteropathy[1]. It develops due to an inadequate immune response to gluten proteins in genetically predisposed patients[1,2].

The only treatment currently available in CD is the lifelong adherence to a strict gluten-free diet (GFD) by dietary exclusion of gluten proteins from wheat, barley, rye, oats and hybrids of these cereals such as triticale and its derivatives[3]. Compliance with the GFD leads to remission of symptoms within a few days or months[4] and normalization of serological tests within 24-36 months[5]. However, mucosal healing in adult CD patients may require a longer time, as described in a study in which recovery of intestinal villi was present in only 34% and 66% of patients after 2 and 5 years, respectively, from the initiation of the GFD. On the other hand, the correct performance of a GFD is difficult to achieve due to multiple factors such as the ubiquity of gluten in the food industry[7], the lack of knowledge of gluten-containing foods, the difficulty in the correct interpretation of product labeling, its high cost or the need not to feel different in sociocultural events, all of which favors frequent exposures to gluten[8]. The rates of non-adherence to the GFD vary according to the study population and the methodology used. According to adherence questionnaires, determination of serological tests or GIP in faeces and urine, variable percentages of non-adherence to the diet have been described, ranging from 9-69% in adults and 14-64% in adolescents[7]. Considering age, adolescents are the group most at risk of deliberately not adhering to the diet for fear of social stigmatization[9,10]. Therefore, rigorous follow-up during the transition to adulthood is essential for raising

awareness of the disease and of the responsibility of adherence to the GFD[9-13]. Lanzini[14] et al. performed 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), and it was observed that only a minority (8%) had normalized intestinal mucosa, and in 27% the histological lesion was unchanged or even worsened[14]. Schiepatti[15] et al. identified as predictors of persistence of villous atrophy age over 45 years at the time of diagnosis, clinical presentation (chronic diarrhea with symptoms and signs of malabsorption), absence of clinical response to GFD, and poor adherence to the diet. This lack of adherence to the GFD was the most important predictor (OR 49.3 CI 95% 26.3-92.2)[15]. Similarly, most studies consider that food contamination and inadvertent exposure to gluten play a fundamental role in this lack of progression towards mucosal healing[14,16-18]. It is also estimated that the average gluten exposure of many patients can exceed 100 mg/day, with some individuals exceeding 600 mg/day, which is sufficient to cause persistent symptoms, villous atrophy and long-term complications[19,20].

The persistence of villous atrophy and inflammation is associated with greater morbidity and mortality, with a significantly lower time free of complications and survival than in patients who achieve mucosal recovery. It is also the main risk factor for the development of complications (HR 9.5 CI 95% 4.77-19.4)[15], such as fractures related to osteoporosis, anaemia and other nutritional deficits, infertility and intestinal neoplasms, especially intestinal lymphoma[1,5,6,21]. Thus, compared to the general population, patients with CD and persistent villous atrophy have 3.78 (CI 2.71-5.12) times the risk of intestinal lymphoma, compared to 1.5 (CI 0.77-2.62) for those with mucosal recovery[5,22].

In summary, the benefits of adherence to the GFD in the reduction of gluten-induced inflammation and its effects on the different organs determine an improvement in the quality of life and a lower risk of complications[5]. Strict monitoring of adherence to the GFD is therefore advisable.

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

Monitoring adherence to the gluten-free diet

Adherence to the GFD is reinforced by regular follow-up of the patient with CD[1,23]. It is established that the frequency of controls should be 3-6 months in patients with a recent diagnosis, and then annually or biennially, indefinitely, once the patient is stable, without symptoms, with serologic normalization and adequately performing the GFD[1,5,24]. However, this follow-up schedule does not prevent there being a high percentage of patients who do not adhere correctly to the GFD, as previously mentioned. [23]Garzón-Benavides et al. carried out a strict follow-up with four check-ups over a year in 94 patients on GFD for at least 24 months. At each visit, a clinical assessment, serological determination, adherence questionnaire and urinary GIP determination were performed, and a significant reduction was observed in the percentage of patients with GIP detection and persistent villous atrophy at the end of follow-up. A relationship was also demonstrated between the frequency of GIP detection and histological lesion.

These results led to the suggestion that follow-up at more frequent intervals, instead of the annual follow-up that had been performed according to clinical practice, improved adherence to the GFD, with the consequent histologic improvement[23]. Thus, regular follow-up at shorter intervals could lead to better adherence to the GFD. The key aspects of periodic medical follow-up are to assess the resolution of symptoms, mucosal healing, the possible appearance of complications, improvement in quality of life and to monitor compliance and adherence to the GFD[5].

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

Clinical and nutritional evaluation

As has been mentioned, one of the objectives of the GFD is the resolution of symptoms and improvement of intestinal absorption, although the GFD itself has limitations in the supply of certain nutrients[1] which is why regular clinical and nutritional assessment is essential in the follow-up of these patients.

In this regard, patients diagnosed with CD must receive comprehensive education on how to follow a strict GFD. This task should be carried out through a multidisciplinary

approach, involving structured educational and training sessions led by healthcare professionals with specific expertise in coeliac disease and gluten-free dietary management. Depending on the organization of the healthcare facility and the availability of trained personnel, this role may be fulfilled by senior dietetic technicians, physicians, dietitians-nutritionists, nurses, or other qualified staff. Nutritional education and dietary intake assessment are essential components of patient care and should include counselling on maintaining a healthy gluten-free diet; an individualized treatment plan tailored to the patient's symptoms and comorbidities; ongoing assessment of dietary intake during follow-up; and provision of relevant online resources. Additionally, information on coeliac patient associations should be provided, as they play a valuable role in supporting adherence and promoting social integration.

However, clinical evaluation alone should not be considered a reliable indicator of mucosal recovery, given the limited value of clinical symptoms as predictors of villous atrophy[25], which may 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, it is not expected that this correlation will improve during follow-up when the patient is on a GFD[29,30]. Thus, it is not possible to use clinical response as an indicator of adherence to the diet and mucosal recovery in asymptomatic or paucisymptomatic patients at diagnosis[31]. On the other hand, more than 70% of patients with persistent villous atrophy 2 years after starting a GFD are asymptomatic[28]. This demonstrates the limited value of clinical evaluation in the assessment of mucosal healing. Additionally, throughout follow-up, patients may present with gastrointestinal symptoms similar to those of CD. It is necessary to discern whether these are due to recurrent gluten exposures secondary to poor adherence to the GFD, to other associated entities, or to functional mechanisms that may be motivated in part by modifications in the amounts of fiber involved in the GFD[32]. Therefore, it is essential to perform a 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). But this assessment is of limited use as a tool for monitoring adherence to the GFD.

Table 1 shows the key aspects of clinical and nutritional assessment and screening for complications in patients with CD.

Serology of coeliac disease

Serology is a frequently used marker for monitoring adherence to the GFD. It is well known that CD antibodies are of great value in the diagnosis of the disease because of their high diagnostic accuracy, with specificity, positive predictive value (PPV), negative predictive value (NPV) of anti-tissue transglutaminase type 2 (anti-TG2) IgA and endomysial antibodies (EMA) approximately 98%, 72%, 99%, and 99%, 83% and 99%, 83% and 99%, respectively[33,34]. However, they are poor predictors of dietary transgression[20,35,36] and have low sensitivity for the detection of villous atrophy at 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 the GFD[14]. There are numerous studies that show that serology, once negative, does not become positive again in a large proportion 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 tests[23].

Therefore, the usefulness of serology in monitoring is reduced to the first months after initiating GFD, such that its decrease until it becomes negative orients the reduction of gluten consumption, but once they have normalized, they are not capable of detecting gluten exposure in low amounts[38] or identifying the persistence or recurrence of villous atrophy[23]. Therefore, the value of serology in long-term follow-up in these patients is very limited.

Structured questionnaires and dietary records

The review of the GFD supported by questionnaires that evaluate adherence and frequency of consumption of certain foods reported by the patient is a tool for detecting gluten consumption, and through them, promoting education towards an adequate diet[1,24]. There are different adherence questionnaires, such as Biagi's or Leffler's Celiac Dietary Adherence Test (CDAT), the latter being the only one translated and validated in Spanish, which allows a rapid evaluation with 7 questions that assess: CD symptoms, self-efficacy expectations, reasons for maintaining the GFD, knowledge of this pathology, associated risk behaviours and the perceived degree of adherence[39,40]. However, these structured questionnaires are subjective and cannot identify

unintentional transgressions that the patient cannot detect, since it has been reported that 30% of patients consume gluten unintentionally and 20% are unable to identify the transgression[41]. On the other hand, they have low sensitivity in the detection of villous atrophy, with a sensitivity of 55% and 25-33% having been reported for the CDAT and Biagi questionnaires, respectively, so their applicability in clinical practice is limited[5].

Assessment by a CD dietitian is highly valuable in identifying failures in dietary knowledge or risk practices for inadvertent gluten exposures. Currently, there is no standardized tool available that allows the expert dietitian to objectively assess compliance with the GFD[42]. Dietary registries such as the standardized dietary evaluation (SDE)[43] and the DIET-GFD related to the risk of gluten exposure and estimated consumption[42] have been developed. However, their external validity has not been demonstrated[10,42]. The lack of objective tools makes it necessary to accurately record the patient's dietary habits (method of food preparation, ingredients of prepared dishes, containers used, brands of commercial products, restaurants, food stores) and other issues related to cross-contact in the patient's medical record, which would allow us to know whether the patient identifies and avoids the sources of gluten exposure[10].

On the other hand, the CD specialist dietitian has a key role in promoting healthy eating, expanding alternative nutritious food choices and discouraging unnecessary restrictive dietary practices[1,3,5]. In this way, micro- and macronutrient deficiencies that may occur during treatment, as well as constipation, can be avoided. Constipation is frequent in these patients due to the low fiber content of GFD, and it may be necessary to supplement the diet with other fiber-rich foods[5].

Very few health care units have specialized nutritionists or dietitians, which is a major barrier in the proper teaching and guidance of the diet in these patients.

Intestinal biopsy

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

The guidelines of the main scientific societies only recommend performing an intestinal biopsy 1-2 years after starting a GFD to verify mucosal recovery, which is especially important in those patients with a higher risk of presenting persistent villous atrophy, such as those diagnosed at an advanced age (over 40 years) or who present 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 create frustration in those patients who perform the GFD correctly and cause excessive concern about gluten exposure, leading to anxiety and depression[47]. Therefore, a more advisable option may be to perform endoscopy 2 years after starting the GFD. It should always be performed on a case-by-case basis in patients with persistent symptoms or nutritional deficits at any time during the course of the disease, and in those in whom the persistence of duodenal histological lesions should be assessed[5]. Given the importance of the histological result in the correct interpretation of adherence and response to the GFD, it is essential to improve the quality of the duodenal biopsies, avoiding poor representativeness. Therefore, a total of 4-6 biopsies (2 of them from the bulb) should be taken one at a time, trying to ensure correct orientation of the samples and avoiding shaking the forceps inside the container[48].

Likewise, a correct histological evaluation by pathologists with expertise in digestive pathology is essential, where the report should include protocolized information on the adequacy of the sample, the increase in intraepithelial lymphocytes, and the crypt ratio (villus and the degree of atrophy)[49,50].

As previously discussed, histologic evaluation should be considered in addition to 2 years after initiating the GFD when there is no adequate clinical response to the GFD. However, it is known that persistent villous atrophy frequently occurs in asymptomatic patients[28]. It is therefore essential to identify patients at high risk of histological lesion by means of non-invasive tools.

Gluten immunogenic peptides in faeces and urine

The determination of GIP in human samples (faeces and urine) is considered a useful tool in the monitoring of adherence to the GFD[1,17,24]. GIP are gluten fragments resistant to gastrointestinal di-management, and the main responsible for the immune response

in patients with CD[51,52]. The recovery of measurable amounts of GIP in feces or urine directly indicates that gluten has passed through the digestive tract and thus has been consumed, thereby non-invasively demonstrating voluntary or involuntary gluten consumption with high sensitivity and specificity[13,19,20,20,35,53]. GIP are eliminated with the faeces, although another part can cross the basolateral membrane of the enterocytes, pass into the portal circulation, reach the kidneys and after a process of ultrafiltration be partially or totally excreted in the urine[54,55]. The determination of these peptides in faeces is performed by enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassay (LFIA) techniques and in urine by LFIA, which allows a 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 faeces varies between 1 and 3 days, with a maximum detection time of 7 days. In urine, the first 3-9 h after ingestion are those with the highest concentration of GIP, and although the probability of detection decreases thereafter, its presence has been described in some cases up to 36 h post-consumption[58].

Multiple studies with different methodologies have compared the determination of GIP in faeces and urine with clinical manifestations, adherence questionnaires and serological tests, demonstrating the greater capacity of GIP in detecting gluten exposure in the diet[20,35,59-63]. Thus, Fernández-Bañares et al.[28] followed 72 adult patients with *de novo* CD for 2 years using clinical and serological assessment, adherence questionnaires and GIP determination in faeces. They observed that 68.4% of the patients showed good or excellent adherence to the GFD according to the questionnaires. However, 53% of the total patients persisted with villous atrophy 2 years after starting the GFD, 72.5% of them had no symptoms and 75% had negative serology. However, in 77% of patients with persistent villous atrophy, GIP was detected in at least one faeces sample[28]. Similarly, Ruiz-Carnicer et al.[27] analysed the clinical usefulness of GIP determination in urine for monitoring adherence to the GFD and its usefulness as a predictor of duodenal histologic lesion, by correlating the punctual determination of GIP with the degree of duodenal histologic lesion. They demonstrated that the measurement of GIP in 3 urine samples over a 7-day period, including the weekend, was the best option for confirming adherence to the GFD due to the high sensitivity (94.4%) and NPV (96.8%) values obtained in relation to duodenal biopsy findings[27].

Subsequently, in the work of Garzón-Benavides et al.[23] demonstrated the relationship between the serial determination of GIP in urine (6 samples over a year) and histologic evolution was demonstrated. Thus, in those patients with histologic normality or mucosal recovery, the detection of GIP in urine decreases during follow-up. In contrast, in those with persistent villous atrophy, the percentage of patients with GIP detection is higher and does not change during follow-up. This shows that frequent detections of GIP, even in small concentrations, tend to 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 lesion, and likewise, the repeated absence of GIP in 2 or more repeated visits over a year with the absence of histological lesion[23]. According to these results, serious determination of GIP in the long-term follow-up of the patient with CD provides guidance on adherence to the GFD and the degree of duodenal histologic lesion. Thus, the best strategy for monitoring adherence to the GFD seems to be the semiannual determination of GIP in faeces or urine.

Previous studies have shown that, in real-life settings, some patients with coeliac disease who occasionally consume gluten may not experience significant clinical or histological damage, which supports the existence of immunotolerance phenomena[64,65]. Although individual tolerance to gluten may vary, the persistence, not the amount, of exposure appears to play a greater role in mucosal damage. Therefore, an isolated positive GIP result should not be interpreted as evidence of histological injury, but rather as an opportunity to prevent future inadvertent exposures.

It should be noted that the use of GIP testing is not indicated in patients who voluntarily report gluten consumption or in those in whom gluten exposure is clearly identified during clinical history taking or dietary assessment. However, GIP testing becomes particularly useful during follow-up, once dietary education has been reinforced, as an objective tool to confirm the cessation of inadvertent gluten exposure.

OPERATIONAL DOCUMENTS

Protocol for the management of GIP in the monitoring of CD (Figure 1).

A number of considerations for optimising sample collection are initially described:

1. **Faeces samples**

- It is recommended that two faeces samples be collected 2-3 days apart during the week prior to the medical examination, including one day during the week and one day reflecting the weekend intake. Thus, different collection schedules are possible: preferably Monday-Thursday or Tuesday-Saturday, and if this is not possible due to the patient's lifestyle or eating habits, another possibility would be, for example, Wednesday-Sunday.
- The collection of one of the samples reflecting weekend intake is due to the fact that gluten exposure is more likely to occur when eating out. This scheme can be adapted according to the patient's lifestyle.
- The patient shall be considered as not exposed to gluten in the one-week assessment if GIP is not detected in either of the two faeces samples. Conversely, the patient is exposed to gluten if GIP is detected in at least one of the two samples.

2. Urine samples

- It is recommended that 3 urine samples be collected during the week prior to the medical examination, 2 mid-week samples and one sample reflecting the weekend intake, with the same proposed aim as for faeces samples. Therefore, there are several possible urine collection schemes, but at least one weekend sample should be included. Thus, a possible schedule could be Monday-Wednesday-Saturday evening or Sunday morning. This schedule can be adapted according to the patient's lifestyle.
- When choosing the optimal time for urine collection, it is recommended to collect the first urine in the morning because 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 in the 6 hours prior to urine collection, as the period with the highest percentage of GIP detection is 6-9 hours post-ingestion.
- The patient shall be considered as not exposed to gluten at the one-week point-in-time assessment if GIP is not detected in any of the 3 urine samples and shall be considered as exposed to gluten if it is detected in at least one of the 3 samples.

The scheme for collecting GIP in faeces and urine samples in the diagnosis and long-term follow-up of CD patients is shown below (Figure 2).

Performing a GIP determination at the time of diagnosis will help to identify those patients who reduce gluten consumption prior to duodenal biopsy, which will allow the histological results to be interpreted correctly. For this purpose, the collection of a single urine (morning) or faeces sample on the same day or the day before the duodenal biopsy is recommended[66-68]. In individuals newly diagnosed with CD who initiate the GFD, a first review could be performed 3-6 months after diagnosis, given the difficulty in adaptation and learning that the GFD involves in the first year after CD diagnosis. The joint performance of GIP and serology at the start of follow-up allows for guidance and orientation of patients and specialists on adherence to the GFD. Thus, a decrease in antibody levels accompanied by the persistence of GIP in most faeces or urine samples will indicate a decrease in gluten intake, but will not ensure correct adherence, so we must reinforce it and resolve any doubts the patient may have about its correct implementation. On the other hand, if the GIP determinations in faeces or urine are negative, the patient has adhered correctly to the diet, and the decrease in the levels of antibodies is within its natural course until normalisation, eliminating the possible anxiety and stress for the patient as the antibodies have not yet become negative.

In patients with well-documented CD who are on a GFD, six-monthly GIP determinations should be performed. In asymptomatic patients with normalised CD serology, mucosal recovery and absence of GIP in successive medical check-ups over 24 months, annual follow-up could be performed, shortening the GIP interval if there is a change in the patient's clinical situation before the annual check-up.

Interpretation of long-term results

- The absence of GIP at subsequent visits (no detection of GIP in any of the samples during the year) will indicate the patient's adequate adherence to the diet.
- The detection of GIP in any of the samples collected will indicate that the patient has been exposed to gluten. The frequency of GIP detection throughout the follow-up will provide insight into the frequency with which the patient is

exposed to gluten and the likelihood of duodenal histological lesion. It has been reported that the presence of more than 4 urine samples over 1 year with GIP detection predicts histological lesions with a specificity of 93%. In summary, the detection of GIP will indicate the need to reinforce adherence to the GFD, preferably in specialised dietary consultations.

- In case of clinical non-response to the GFD, serial determination of GIP will make it possible to discern whether this is due to poor adherence to the diet or whether, on the contrary, adherence is correct (evidenced by the repeated absence of GIP at successive visits). In this case, it should be assessed whether the symptoms are due to the coexistence of other clinical entities (Table 1).
- If, after the relevant tests, no associated pathology is found to justify the persistence of symptoms or analytical alteration, it is recommended that a new duodenal biopsy be performed:
    - The histological normality and the repeated absence of GIP would point to the functional origin of the symptoms.
    - The persistence of villous atrophy makes it necessary to assess whether the diagnosis of CD was correct (Table 2), whether there are other causes of villous atrophy (Table 3) or the development of refractory coeliac disease (RCD).

RESOURCES NEEDED

The clinical use of GIP testing as a tool for monitoring adherence to the gluten-free diet should be conducted by experienced personnel within the framework of a specialised Coeliac Disease Unit[69-71].

This is a protocol that aims to guide clinical care and diagnostic and therapeutic decision-making, in accordance with the determination of GIP, in monitoring adherence to the GFD in patients with CD. The minimum resources for the development of the protocol are specified below.

- Local
- Staff
- Clinical-diagnostic material
- Financial resources

Local

This protocol applies to the primary care setting with physicians specialised in CD, and in general or monographic gastroenterology practices oriented to the management of patients with CD and other gluten-related pathologies. This care can be provided in public or private healthcare facilities.

Staff

Centers adhering to the protocol should have gastroenterologists, biochemists, immunologists, pathologists with expertise in digestive pathology and, where possible, dieticians or nutritionists specialised in CD.

Clinical-diagnostic material

The application of the protocol requires:

1. Laboratory material for basic study of:

a) Hematometry
b) Biochemistry
c) Immunology
d) Genetics
e) Microbiology

2. Laboratory material for the study of GIP in faeces or urine. Kits for rapid detection of GIP in faeces or urine by LFIA are required, as well as usual laboratory material (urine collection bottle, 100 µL - 1000 µL pipette, disposable tips, Eppendorf® type plastic vials, 96-100% ethanol, only in the case of faeces, and powder-free gloves). In the case of using the faeces ELISA technique, the ELISA kit optimised for the detection of GIP will be required, as well as the usual laboratory equipment mentioned above. A plate reader with 450 nm filter, vortexes, thermostatic or 50 °C adjustable bath, a multichannel pipette and an automatic plate washer are also required.

3. Digestive Endoscopy Unit with the appropriate equipment to perform upper endoscopy and other services if necessary (colonoscopy, enteroscopy and video capsule endoscopy).

4. Anatomical pathology laboratory with pathologists with expertise in digestive pathology to correctly identify and define the histological lesions associated with CD.

5. Facilitate access to consultation with Dietitian-Nutritionists with expertise in CD to identify possible sources of gluten exposure and make appropriate modifications to the GFD.

6. Resources needed in the identification of other pathologies:

- **a)** Functional digestive explorations laboratory for the performance of:
  - i) Hydrogen breath test for intolerance to sugars (lactose, fructose, sorbitol)
  - ii) Small intestinal bacterial overgrowth
- **b)** Faecal elastase or 13C-triglyceride respiratory test for the study of exocrine pancreatic insufficiency.
- **c)** Radiodiagnostic Service to perform computerised axial tomography or Magnetic Resonance Imaging (MRI) of the abdomen, MRI enterography.

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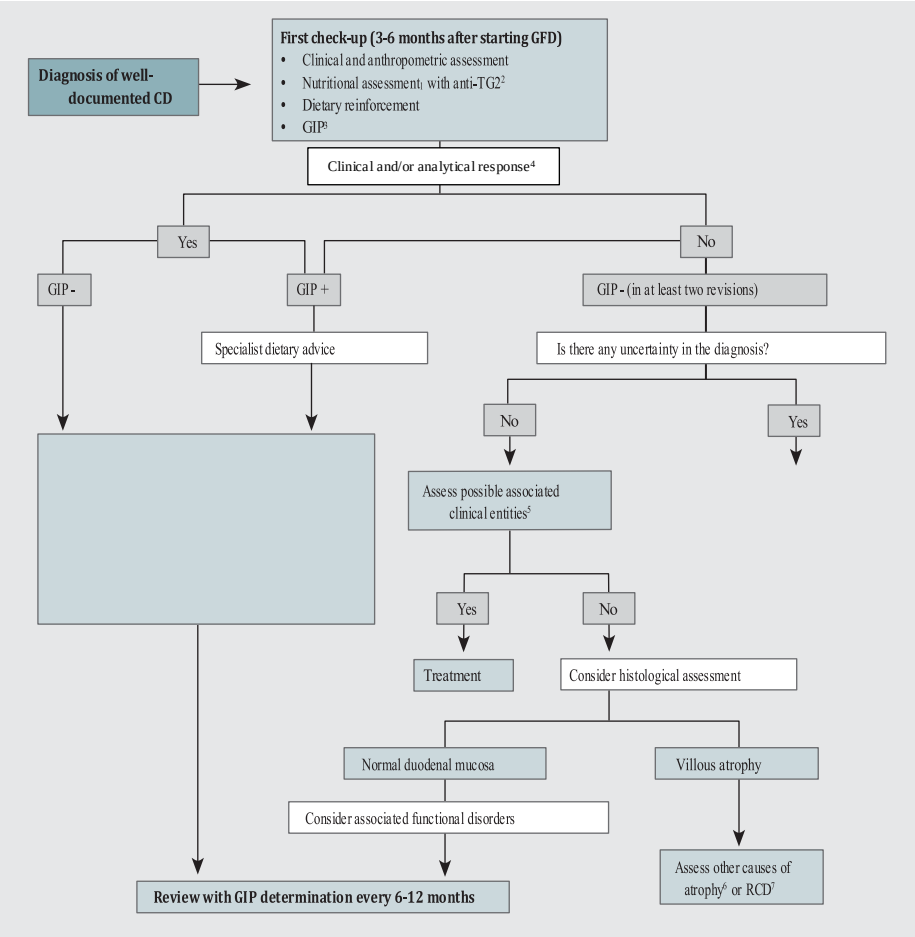
FIGURES AND TABLES

Figures` legends:



[1]Hemogram, general biochemistry, thyroid hormone, coagulation, iron metabolism, calcium, phosphorus, magnesium, vitamin D3, folate and cobalamin (B12). In the presence of a classic presentation pattern (malabsorptive diarrhoea and weight loss) or in the presence of severe watery diarrhoea, consider determining levels of copper, selenium, zinc and vitamins: A, E, K, riboflavin (B2), niacin (B3), pyridoxine (B6), biotin (B7). Evaluate the periodic determination of these micronutrients in patient follow-up when there are doubts about whether the GFD is nutritionally complete and balanced. [2]Determination of anti-TG2 antibodies until negative. [3]Collection of 3 urine samples or 2 stool samples according to the proposed scheme. At each check-up, if one of the samples is positive, the patient shall be referred to a specialised dietetic consultation. If all the samples are negative for GIP determination, the established review schedule shall be followed. [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 salt malabsorption, irritable bowel syndrome. [6]Autoimmune interopathy, common variable immunodeficiency, Crohn's disease, eosinophilic gastroenteritis, mastocytosis, drugs (olmesartan), parasitosis (e.g. Giardiasis), other infections (e.g. tuberculosis), Whipple's disease, abetalipoproteinaemia. [7]Consider if symptoms of malabsorption and villous atrophy persist 12 months after starting GFD.

Abbreviations: CD, coeliac disease; GFD, gluten-free diet; anti-TG2, anti-tissue transglutaminase 2 antibodies; GIP, gluten immunogenic peptides; RCD, refractory coeliac disease.

Figure 1: Algorithm for the follow-up of coeliac disease.

Figure 2: Scheme for collecting GIP in faeces and urine samples in the diagnosis and long-term follow-up of CD patients is shown.

Table 1: A. Key aspects of clinical and nutritional assessment and screening for complications in patients with CD during follow-up**. B.** Clinical entities associated with coeliac disease that may justify the persistence of symptoms despite a gluten-free diet.

CLINICAL ASSESSMENT	NUTRITIONAL ASSESSMENT	DEVELOPMENT OF COMPLICATIONS
Dyspepsia	Weight, height and BMI	Osteopenia / Osteoporosis
Meteorism	Blood count	Infertility
Diarrhoea	General biochemistry Coagulation	Autoimmune hepatitis
Abdominal pain	Iron metabolism Thyroid hormones Calcium, phosphorus, magnesium, folate, selenium, ferritin, folic acid, vitamin D, and zinc and vitamins A, D, E, K, B complex vitamins.	SI lymphoproliferative disorders / Other neoplasms
Astenia		RCD

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

B)

CLINICAL ENTITY	DIAGNOSTIC TEST
Small intestinal bacteral overgrowth*	Hydrogen breath test (glucose, lactulose)
Microscopic colitis	Step biopsies of the colon
Exocrine pancreatic insufficiency	^{13}C labeled triglyceride breath test Faecal elastase
Crohn's disease	Biomarkers of inflammation: CRP, ESR, FCP MRI Enterography Colonoscopy with Ileoscopy
Bile acid malabsorption	Bile acid malabsorption scan (SeHCAT) Therapeutic trial with resincholestyramine: 8 g for 10 days
Irritable bowel syndrome	Rome IV criteria Exclusion of other pathologies

Abbreviations: *^{13}C, Carbon 13; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; FCP, faecal calprotectin; MRI, magnetic resonance imaging; SeHCAT, selenium 75-labeled taurocholic acid scintigraphy (modified from Project Prodiggest: Diagnostic evaluation of the patient with clinically suspected celiac disease and seronegative villous atrophy, 2020, from the original Spanish title "Evaluación diagnóstica del paciente con sospecha clínica de enfermedad celíaca y atrofia vellositaria seronegativa").* If the patient has a predisposing factor is present (surgery, stenosis, motility disorders, ….)*

Table 2: Criteria for the assessment of a correct diagnosis of coeliac disease.

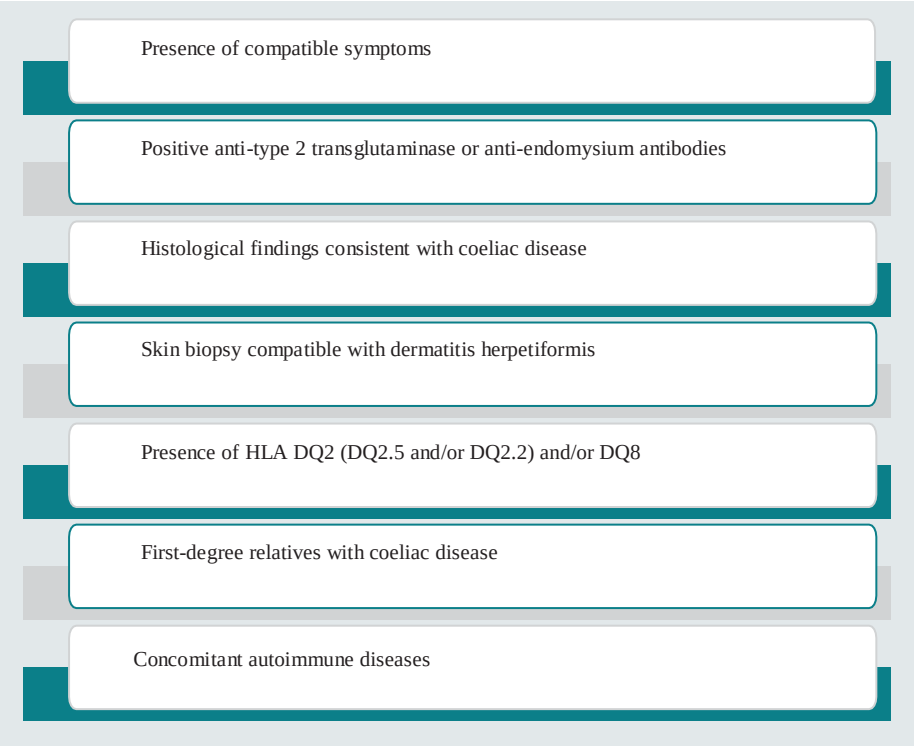


Table 3: Main causes of non-celiac villous atrophy and features that help in the differential diagnosis.

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CLINICOPATHOLOGICAL ENTITY	CLINICOPATHOLOGICAL FEATURES THAT ARE HELPFUL IN DIFFERENTIAL DIAGNOSIS
Immune-mediated	
Crohn's disease	Typical endoscopic appearance. Histological presence of transmural inflammation, non-caseating granulomas, fibrous tracts and altered crypt architecture.
Food allergies	Relationship between symptoms and specific foods. Positivity of serological tests - IgE 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-enterocyte antibodies. Heterogeneous pattern of small intestinal lymphocytic infiltration.
Graft-versus-host disease	History of organ transplantation
Common variable immunodeficiency	Low immunoglobulin levels. Respiratory and other organ infections. Absence of plasma cells in lamina propria.
Microbial	
Tropical sprue	Travel to endemic areas (Caribbean, South India, South-East Asia)
Tropherima whipplei (Tw)	PAS-positive macrophages. Demonstration of Tw DNA by CRP
Mycobacterium tuberculosis	Mucosal granulomas. Quantiferon positive
Drugs	
NSAID	History of NSAID use
Olmesartan, candesartan	History of HTN with use of these drugs
Neoplasms	
Immunoproliferative small intestinal disease (IPSID)	Dense infiltration of plasma cells in the lamina propria. Presence of aberrant lymphocytes in the study of lymphoma.
Lymphoma	Histological lesions compatible with lymphoma on histological examination
Metabolic and degenerative diseases	
Abetalipoproteinaemia	Practically limited to childhood. Histological demonstration of intracytoplasmic vacuoles.
Lymphangiectasia	Intestinal villi slightly widened. Acellular mass, displacement through lymphatic ducts.
Amyloidosis	Amyloid deposition in the mucosa (Congo Red stain)
Mastocytosis	Mast cell infiltration (toluidine blue)
Others	
Collagenous sprue	Mucosal atrophy and excessive subepithelial collagen deposition

Abbreviations: *PAS, Periodic Acid-Schiff stain; PCR, polymerase chain reaction; NSAID, non-steroidal anti-inflammatory drug; HTN, hypertension (modified from Project Prodiggest: Diagnostic evaluation of the patient with clinically suspected coeliac disease and seronegative villous atrophy, 2020, from the original Spanish title "Evaluación diagnóstica del paciente con sospecha clínica de enfermedad celíaca y atrofia vellositaria seronegativa")*