The Spectrum of Gluten-Related Disorders

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Abstract Gluten is a rubbery mass consisting of water insoluble proteins from wheat, rye and barley. It is well known that dietary gluten may trigger an abnormal immune response in genetically susceptible individuals, causing a number of disorders whose clinical spectrum ranges from celiac disease to dermatitis herpetiformis and wheat allergy. In the last few years, other conditions that are supposed to be pathogenetically linked with gluten have emerged, such as gluten ataxia and non-celiac gluten sensitivity. In this review, we critically evaluate clinical and diagnostic aspects of gluten-related disorders.

Keywords Celiac disease · Dermatitis herpetiformis · Gluten ataxia · Non-celiac gluten sensitivity · Wheat allergy

Introduction

Gluten is defined as a complex of water insoluble proteins from wheat, rye and barley [1]. These proteins are well known to be the environmental factors that are harmful to patients affected by celiac disease, its complications, dermatitis herpetiformis and wheat allergy. Recently, other conditions that could have a pathogenetic link with gluten

have emerged, such as gluten ataxia and non-celiac gluten sensitivity. The aim of this paper is to review these conditions.

Celiac Disease

Celiac disease is a chronic enteropathy, caused, in genetically predisposed individuals, by gluten intake in the diet [2]. One of the typical characteristics of this disease is damage to the small bowel mucosa, which, starting from the duodenum and extending distally, reduces the absorption surface, thus causing varying degrees of malabsorption. Celiac lesions improve when gluten is eliminated from the diet.

Epidemiology

Thanks to the introduction of modern antibody screening tests, it has been possible to demonstrate that celiac disease is a very frequent condition in Caucasian populations, both in adults and in children [3•]. The prevalence of celiac disease in the general Italian population has been estimated at around 1/150 [4]. Very similar results have been obtained in other Western countries [5]. However, in first-degree relatives of celiac patients, as well as in a series of pathological conditions that may be associated with celiac disease (see “Clinical Picture” section), its prevalence may be over 15 % [6]. Finally, in patients with severe global malabsorption, the prevalence of celiac disease may even be as high as 40–50 %. Awareness of these epidemiological aspects is crucial, since the diagnostic strategy should be based on the risk the patient has of being affected by celiac disease.

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Etiology

It has been known for some 60 years now that the environmental triggering factor of celiac disease is gluten. Gluten comprises two main protein fractions: gliadins and glutenins [1]. Gliadins, undoubtedly toxic, are alcohol-soluble and can be differentiated by means of modern electrophoretic techniques into more than 100 components, grouped in turn into four main types (ω 5-, ω 1,2-, α/β -, and γ -). Prolamins, i.e. the alcohol-soluble components of barley (hordein), rye (secalin) and oats (avenin)—the toxicity of the latter still being doubtful [7]—are similar to gliadins in their amino acid composition. Glutenins, which can be further divided into high and low molecular weight subunits, are alcohol-insoluble and their toxicity has recently been confirmed [8].

Gluten is characterized by a unique amino acid composition, rich in glutamine and proline. It is precisely these two amino acids that give gluten its particular properties, such as the tendency to bind to HLA class II molecules, the resistance to breakdown by luminal proteases, and the rigid α -helix conformation. The complete degradation of gliadins to single amino acids deprives them of their toxicity, while digestion by means of pepsin and trypsin generates peptides that are still toxic. Two oligopeptides of α -gliadin have recently been discovered, 31–49 and 51–70, that are capable of flattening the intestinal mucosa both in vivo and in vitro. The oligopeptide 51–70 has also been shown to be capable of activating gliadin-specific and HLA-DQ2–restricted T lymphocytes, which play a crucial pathogenic role in the generation of celiac lesions. Finally, the oligopeptide 57–73 of α -gliadin, which is very similar to 51–70, is the peptide towards which the first immune response is triggered.

The importance of genetic factors in the pathogenesis of celiac disease is demonstrated by studies carried out on relatives of celiac patients. These studies have shown a prevalence of 10–15 % in first-degree relatives and of 30 % in HLA-identical relatives. The concordance for disease between monozygote twins, which is much greater than that between HLA identical siblings, confirms that non-HLA genes are also implicated in the pathogenesis of this condition. The association between celiac disease and HLA molecules is very strong [9]. In fact, more than 90 % of celiac patients are carriers of the HLA-DQ2 molecule encoded by the alleles HLA-DQA1*0501 and HLA-DQB1*0201. Most of the remaining patients who do not present the DQ2 molecule express the DQ8 molecule encoded by the alleles HLA-DQA1*0301 and HLA-DQB1*0302. It should, however, be clarified that these alleles represent a condition that is necessary, but not sufficient, for the development of this disease, as confirmed by their presence in 25–30 % of the general population.

It is known that tissue transglutaminase represents the autoantigen toward which the anti-endomysial antibodies

are directed [10, 11]. In addition, the importance of tissue transglutaminase in the pathogenesis of celiac disease is also demonstrated by the fact that, of the various proteins, gliadin is one of its preferential substrates [11]. By specifically deamidating the glutamine residues, of which gliadins are particularly rich, tissue transglutaminase increases the binding affinity between gliadins and HLA-DQ2 and HLA-DQ8 molecules [12, 13]. Thus, deamidated gliadin is presented by antigen presenting cells to T cells in a form that more effectively determines its activation [14].

Pathogenesis

Numerous demonstrations confirm that HLA-DQ2/DQ8–restricted antigen presenting cells (i.e. macrophages and dendritic cells) present gliadin, which has penetrated the intestinal wall by the transcellular or paracellular route [15–18], to the T cell receptor expressed on the surface of the CD4⁺ lymphocytes in an unmodified or, more frequently, deamidated form. The consequent T cell activation leads to hyperproduction of mucosal pro-inflammatory Th1 cytokines, including interferon- γ [19], which, together with the direct cytotoxic activity exerted by the CD8⁺ intraepithelial lymphocytes [20] and the activation of matrix metalloproteases [21], induce a dramatic acceleration enterocyte apoptosis, causing villous atrophy and cell hyperproliferation with crypt hyperplasia-hypertrophy.

Although there are no doubts regarding the fact that cell-mediated immunity plays a very important role in the pathogenesis of celiac disease, considerable evidence suggests a joint role of humoral immunity. In untreated celiac disease, high serum levels of gluten-dependent tissue anti-transglutaminase and anti-gliadin antibodies are found. It has been proposed that the pathogenetic mechanism that leads to the production of these antibodies is triggered by the formation of gliadin-transglutaminase complexes that are also presented to the immune system [11]. Although it is not yet clear whether the role played by humoral immunity is pathogenetically a primary or a secondary role, it is undeniable that the search for these antibodies represents a great diagnostic aid.

Clinical Picture

Celiac disease can present at any time in a subject's life. It is possible that symptom onset takes place at weaning, when gluten is first introduced into the diet. In this case, the symptoms can persist during infancy or can decrease and even disappear completely during adolescence, and then, perhaps, represent during adulthood. If, on the other hand, the symptoms appear directly in adulthood, it is possible that this occurs in concomitance with pathological (bacterial or viral enteritis, surgery) or physiological events

(pregnancy) that can precipitate the appearance of symptoms. In patients diagnosed in adulthood, it is difficult to distinguish between those who developed the intestinal lesions in infancy and those who developed gluten intolerance and intestinal lesions directly as adults.

The symptoms present at onset can vary considerably, and it has been hypothesized that this can depend on the degree of extension of the lesions along the small intestine [22]. A patient with extensive lesions will be more likely to present with a severe syndrome of global malabsorption, weight loss and other deficiency symptoms, steatorrhea and diarrhea. This form of presentation is defined as “classical/major”. A patient in whom the intestinal lesions involve only the proximal part of the small intestine, thanks to the organ’s considerable compensatory ability, may present with a minor degree of malabsorption and have minor, transitory and/or extraintestinal symptoms. This form of presentation is defined as “non classical/minor” and the symptoms that most often characterize this form are listed in Table 1. Finally, patients who do not report any symptoms, and who are diagnosed due to the positivity of one of the antibodies linked to celiac disease are defined as carriers of a “silent” form of celiac disease [2].

Numerous immune-mediated diseases have been observed in association with celiac disease (Table 2). It is very important to be aware of these associations, since their finding can be the only indication of the presence of otherwise undetected celiac disease. In celiac patients with concomitant autoimmune disorders, a gluten-free diet is sometimes beneficial for the associated disease too, due to the better absorption of drugs and the reduction of the intestinal and systemic inflammation.

Diagnosis

The diagnosis of celiac disease is still based on the demonstration of the increase in inflammatory infiltrate and of villous atrophy [23, 24], by means of perendoscopic duodenal biopsies. It is, however, necessary to point out that

Table 1 Signs and symptoms that can, if present to an isolated degree, characterize a “minor” form of celiac disease

Microcytic, macrocytic and normocytic anemia
Stunted growth
Hypertransaminasemia
Bone pain and pathological fractures
Dyspepsia and vague abdominal disorders
Isolated asthenia
Late menarche, amenorrhea, early menopause, infertility, multiple miscarriages
Neurological symptoms (epilepsy, ataxia, peripheral neuropathies)
Alterations to the skin and adnexa (dry, non-elastic skin, clubbing, alopecia)
Hypoplasia of the dental enamel and aphthous stomatitis

the antibody tests have revolutionized our knowledge of the epidemiology and clinical presentation of this condition. The availability of non-invasive tests with a high degree of sensitivity and specificity that are able to guide “oriented” biopsies in subjects who would otherwise not have been biopsied on the basis of symptoms has made it possible to document, on one hand, the true high prevalence of this disease and, on the other, to recognize new clinical forms.

For a correct diagnostic procedure, it is absolutely necessary that the antibody tests and the biopsies are performed while the patient is still ingesting gluten. Both the antibodies and the intestinal lesions are, in fact, gluten-dependent and will only be normalized after removal of gluten from the diet. As a consequence, the search for them will lead to false negative results if the patient has already started a gluten-free diet.

The antibodies specific for celiac disease are the anti-gliadin antibodies and the anti-endomysial and anti-transglutaminase antibodies. The IgG and IgA class anti-gliadin antibodies were the first to have a diagnostic role, but due to the relatively low levels of sensitivity and specificity, they are now considered obsolete. Their use is recommended only in the first 2 years of life, the period in which a reduced sensitivity of anti-transglutaminase and anti-endomysial antibodies has been reported. The latter are both, in fact, directed against the same antigen: the tissue transglutaminase enzyme. However, the search for anti-endomysial antibodies is performed by indirect immunofluorescence using monkey esophagus or human umbilical cord as the substrate, while the anti-transglutaminase antibodies can be quantified with the ELISA method. The sensitivity and specificity of the first are 95 and 100 %. The sensitivity of the tissue anti-transglutaminase antibodies is 98 %, but their specificity is lower than that of the anti-endomysial antibodies [25, 26]. Both are IgA class, and the search for the IgG class is useful only in cases in which celiac disease is associated with a selective IgA deficiency [27].

Table 2 Autoimmune diseases or diseases characterized by immunological alterations observed in association with celiac disease

Dermatitis herpetiformis	Primary biliary cirrhosis
Alopecia areata	Autoimmune thyroiditis
Autoimmune neuropathies	Addison’s disease
Atopy	IgA nephropathy
Systemic lupus erythematosus	Inflammatory bowel diseases
Idiopathic dilated cardiomyopathy	Selective IgA deficiency
Sjögren’s syndrome	Idiopathic juvenile arthritis
Type I diabetes	Poliomyositis
Autoimmune hepatitis	Cutaneous and systemic vasculitis
Sclerosing cholangitis	Down’s syndrome

The duodenal biopsy is the test that confirms a positive serology. At least four biopsies must be taken [28], correctly processed with the villi facing upwards so as to avoid technical artefacts being mistaken for actual pathological alterations. The celiac disease damage consists of villous atrophy, crypt hypertrophy and an increase in inflammatory infiltrate at intraepithelial level and of the lamina propria [24]. None of these lesions are, however, absolutely specific, but can all, including total villous atrophy, be found in other pathological conditions. As a consequence, a confirmed diagnosis of celiac disease can only be made if, in addition to the lesions, the antibody test is positive, or there is a significant regression of the lesions following an appropriate period of strict gluten-free diet. HLA typing can certainly not be used to confirm a diagnosis of celiac disease, since the DQ2–DQ8 haplotype is present in 25–30 % of the general population. However, the negativity of these genes allows the diagnosis of this condition to be excluded [29].

Therapy

The standard treatment of celiac disease is a gluten-free diet. This must be very strict and carefully followed for the rest of the patient's life [30]. This requires not only the elimination of all foods containing wheat, but also those containing cereals taxonomically similar to wheat, such as barley and rye. Oats were considered toxic for patients with celiac disease until the mid 1990s, when a series of studies demonstrated that they were harmless for most patients [31].

Complications of Celiac Disease

Prolonged exposure to gluten, due to a not strict enough gluten-free diet and/or to a late diagnosis, is the most important factor for the development of complications of celiac disease [32]. It is in fact known that the mortality of celiac patients who were diagnosed in pediatric age and have since maintained a strict diet is not greater than for the general population, while for celiac patients diagnosed in adulthood, the mortality rate is doubled and increases sixfold in the event of frequent dietary transgression [32, 33].

The increased mortality, which characterizes celiac disease in adults, is supported by the onset of a series of complications that must be suspected in the event of no clinical and histological response to the diet, or when, despite a strict diet, symptoms such as abdominal pain, fever, diarrhea, hemorrhage and anemia reappear [34, 35].

Intestinal Lymphoma

Intestinal lymphoma is the most frequent and severe complication. This is an enteropathy-associated T cell lymphoma, which often arises between the fifth and seventh decades of life [35]. The clinical picture, in most cases, is characterized by abdominal pain, weight loss, fever and diarrhea, while in other cases intestinal occlusion or perforation may also be present. Intraepithelial lymphocytes in enteropathy-associated T cell lymphoma are mitotically normal, but present an abnormal phenotype ($CD3^-$, $CD4^-$, $CD8^-$, $CD103^+$) and a monoclonal rearrangement of the genes encoding the T cell receptor γ chain [36].

Ulcerative Jejunoileitis

Ulcerative jejunoileitis is a less frequent complication. It is characterized by multiple and transversal ulcers of the small intestine, followed by scarring retraction that frequently leads to stenosis of the affected intestinal section. Histologically, the lesions present an aspecific, non-granulomatous inflammatory infiltrate and varying degrees of villous atrophy of the mucosa adjacent to the ulceration. The clinical characteristics are similar to those of enteropathy-associated T cell lymphoma, with which ulcerative jejunoileitis is often associated [34]. The mortality of ulcerative jejunoileitis is higher than 70 % and is due to intestinal occlusion, perforation and hemorrhage.

Refractory Celiac Disease

Refractory celiac disease is characterized by a duodenal mucosa with severe villous atrophy, despite the gluten-free diet, and by the reappearance of symptoms. An abnormal phenotype of the intraepithelial lymphocytes and/or a monoclonal re-arrangement of the γ chain of the T cell receptor are often present, and they allow refractory celiac disease to be classified into type I (without these features) and type II (with these features) [37]. Patients with refractory celiac disease type II seem to have a higher risk of developing enteropathy-associated T cell lymphoma [38].

Other neoplastic complications of celiac disease include adenocarcinoma of the small intestine and squamous cell carcinoma of the pharynx and esophagus. Non-neoplastic complications include spleen defective function or atrophy [39, 40], potentially responsible for fatal infections sustained by encapsulated bacteria [41], and collagenous sprue characterized by the deposit of thick bands of subepithelial collagen.

Dermatitis Herpetiformis

Dermatitis herpetiformis is a rare gluten-sensitive blistering itchy skin disease strictly connected with celiac disease: gluten-sensitive villous atrophy, and circulating antibodies directed against not only epidermal transglutaminase but also tissue transglutaminase, are present in most patients with dermatitis herpetiformis [10, 42]. Finally, the association with HLA is the same as in celiac disease: 90 % of patients have HLA-DQ2 and almost all the remaining have HLA-DQ8.

Following the consumption of gluten, pruritic maculopapular and vesicular lesions can develop at any age over the extensor surfaces of the major joints, back, buttocks and face. Clinical diagnosis may be difficult, and it is currently made on the basis of a frozen biopsy from perilesional affected skin, processed for direct immunofluorescence [43], demonstrating IgA granular deposits localized either in the dermal papillae or along the dermo-epidermal junction [44]. In the last few years, the antibodies directed against the different transglutaminases were proposed as a tool for the diagnosis of dermatitis herpetiformis [45–48].

Gluten Ataxia

Hadjivassiliou et al. [49•] originally defined gluten ataxia as idiopathic sporadic ataxia in association with positive anti-gliadin antibodies with or without enteropathy on duodenal biopsy. In a series of more than 200 patients with idiopathic sporadic ataxia, they found that almost half of these patients (101 out of 215) had positive anti-gliadin antibodies. Moreover, gluten ataxia was found in 20 % of all the patients with ataxias, 25 % of the patients with sporadic ataxias, and 45 % of the patients with idiopathic sporadic ataxias [49•]. On the other hand, anti-endomysial antibodies and anti-tissue transglutaminase IgA antibodies are present in only 38 and 22 %, respectively, of patients with gluten ataxia. As far as HLA typing is concerned, 70 % of patients with ataxia and positive anti-gliadin antibodies are DQ2-positive, while the remaining 30 % are DQ8 and DQ1 variants positive.

Onset of gluten ataxia is usually insidious, as symptoms develop at a mean age of 53 years [49•]. Most of the times, cerebellar ataxia dominates the clinical picture, but ataxia associated with myoclonus, palatal tremor, opsoclonus or chorea is also possible. Rarely, the ataxia mimics paraneoplastic cerebellar degeneration with a very rapid and progressive course. Gait ataxia is present in virtually all the patients and most of them also have limb ataxia. Gaze-evoked nystagmus and other ocular signs of cerebellar dysfunction are seen in up to 80 % of cases. Although diarrhea, weight loss and other gastrointestinal symptoms are present in only a minority of the patients with gluten ataxia, duodenal

biopsy shows increased intraepithelial lymphocytes and a certain degree of villous atrophy in a third of the patients.

According to Hadjivassiliou et al. [49•], prolonged gluten exposure in patients with gluten ataxia results in irreversible loss of cerebellar Purkinje cells. Response to gluten-free diet therefore depends on the duration of the ataxia, and prompt treatment is necessary to improve or stabilise the ataxia. Although the benefits of a gluten-free diet in the treatment of patients with gluten ataxia was addressed in only a few studies, mainly case reports, most of these reports suggest variable, but overall favourable, responsiveness to a gluten-free diet [49•].

Wheat Allergy

Wheat allergy is an IgE-mediated immunological reaction against wheat proteins. Wheat allergy includes different forms identified on the basis of the route of allergen exposure and the following immune response: respiratory allergy, classical food allergy, wheat-dependent exercise-induced anaphylaxis and contact urticaria [50]. In a systematic review [51], the prevalence of IgE to wheat in adults was reported higher (>3 % in several studies) than for perception (<1 %).

Respiratory allergy, including baker's asthma and rhinitis, is a well-characterized allergic response to the inhalation of wheat and cereal flours and dusts [52]. Classic food allergy, caused by the ingestion of wheat, affects skin, gastrointestinal tract or respiratory tract. Wheat-dependent exercise-induced anaphylaxis is a syndrome induced by the ingestion of wheat and subsequent physical exercise [52]. Contact urticaria is an allergic reaction to wheat, characterized by erythema and then eczema at the site of skin contact with wheat [53].

Skin prick tests and in vitro IgE assays are first-level diagnostics for wheat allergy. However, the positive predictive value of these tests is less than 75 %, mainly in adults, due to the cross-reactivity with grass pollens. Moreover, most commercial reagents for skin prick tests have a low sensitivity as they are mixtures of water-soluble and salt-soluble wheat proteins that lack allergens from the insoluble gliadin fraction. Recently, a wide range of wheat proteins have been identified and characterized as allergens [54]. Some of them are now available for component resolved diagnosis in wheat allergy with an increase in diagnostic accuracy of the in vitro IgE assays.

Non-Celiac Gluten Sensitivity

Recently, a new condition has been reported, termed non-celiac gluten sensitivity, characterized by gluten-dependent intestinal and/or extra-intestinal symptoms in the absence

of both small bowel celiac lesions and positivity for serum anti-endomysial and anti-transglutaminase antibodies [55]. A number of different mechanisms have been implicated in the pathogenesis of non-celiac gluten sensitivity, including the abnormal innate immune response to gluten, starch malabsorption, opioid property of gluten, gluten-induced low-grade inflammation and nocebo effect of the ingestion of gluten-containing foods [56]. In the absence of a reliable biomarker, a randomized double-blind placebo-controlled crossover gluten challenge test remains the method with the best accuracy for diagnosing non-celiac gluten sensitivity in subjects suffering from intestinal and extraintestinal gluten-dependent symptoms, in whom both celiac disease and wheat allergy have been previously excluded. Only three randomized double-blind trials have been performed so far [57–59], all conducted on fairly heterogeneous populations and with results that are difficult to compare due to different study design, different duration, different food used in the active group and different method of masking the food. Moreover, in Biesiekierski's first study [57], the authors did not bother to evaluate a priori the recognisability of the food in blind that contained gluten. Unmotivated gluten-free diets should be discouraged, not only for the costs to the community [60], but also for the risk of misdiagnosing celiac disease.

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Ethical Standards This article does not contain any studies with human or animal subjects performed by any of the authors.

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