Human Immunology xxx (xxxx) xxx

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Review

Diagnosing coeliac disease: A literature review

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ARTICLE INFO

Article history:
Received 4 May 2021
Revised 14 July 2021
Accepted 30 July 2021
Available online xxxx

Keywords:
Coeliac disease
Diagnostics
South Africa
HLA
Autoantibodies
Biomarkers

ABSTRACT

Coeliac disease (CD) is an autoimmune gastroenteropathy triggered by gliadin and gliadin-tissue transglutaminase (tTG) complexes. CD is one of the few autoimmune diseases with an accurate, non-invasive serological test. Anti-endomysial, anti-tTG and anti-deaminated gliadin peptides (DGP) antibodies are currently used for serological tests with tTG ELISAs being the superior test. Duodenal biopsy, although invasive, is the gold standard for CD diagnosis. HLA genotyping and flow cytometry can also be used as supplementary tests.

The incidence of CD is rising globally although the reasons for this remain unclear. In addition, the true incidence of coeliac disease in African populations remains unknown although recent work suggests that South African populations express the alleles associated with this disease.

This review examines the pathogenesis and diagnosis of coeliac disease and considers novel and innovative biomarkers in its diagnosis specifically in an African population.

Contents

1. Introduction

Coeliac disease (CD) is an autoimmune enteropathy with an estimated prevalence of 0.5–1% worldwide and a rising incidence [1,2]. It is one of the commonest autoimmune diseases affecting predominantly Caucasian populations. Recently there has been a

Abbreviations: T1DM, Type 1 diabetes mellitus; DGP, Deaminated gliadin peptide; tTG, Tissue transglutaminase; EMA, Anti-endomysial antibodies; RCD, Refractory Coeliac Disease; GFD, Gluten-free diet.

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https://doi.org/10.1016/j.humimm.2021.07.015
0198-8859/

rise in the incidence of type 1 diabetes mellitus (T1DM) in other population groups, including black and mixed-race patients, some of which have developed CD [3,4]. It has been hypothesized that maize-based diets, popular in South Africa, maybe protective against CD but that recently, with a shift to a more wheat-based diet, increased gluten ingestion may increase the risk of developing CD [5,6]. In addition to gluten ingestion, CD has been associated with several HLA alleles found to be well represented in our South African (SA) population [7]. Despite the potential risk, studies of CD in African populations are small and the prevalence of CD in South Africa is unknown [4].

1.1. Immunopathogenesis of coeliac disease

CD is a CD4 + T-cell mediated inflammatory disorder [2,8]. The CD4 + T cells are specific to a gliadin fragment complexed to tissue transglutaminase (tTG) [1,9–11]. Gliadin (a non-digestible, immunogenic component of gluten) binds with CXCR3 receptors on enterocytes (Fig. 1) [9,12]. The binding transiently increases gastrointestinal tract (GIT) permeability and allows gliadin fragments to move into the lamina propria, triggering an inflammatory response with subsequent intestinal epithelial apoptosis and the release of cellular contents, including the enzyme, tTG [8,13,14]. tTG deaminates positively charged glutamines into negatively charged glutamic acid residues [8,9,15]. The deaminated gliadin and the tTG-gliadin complexes are endocytosed by antigen-presenting cells (APCs), such as macrophages and dendritic cells which present processed forms of these molecules on HLA-DQ2 and -DQ8 to gliadin-specific helper T-cells (TH) causing activation [8,16]. TH2 cells activate B-cells, which produce anti-tTG and anti-gliadin antibodies (Table 1). TH1 cells produce IFN-γ and TNF-α which further increases gut permeability, inflammation and causes an enteropathy which ultimately results in villous atrophy [1]. TH17 cells and the accompanying cytokines have also been associated with an inflammatory role CD [17,18]. CD8 cytotoxic lymphocytes (CTLs) are important contributors to CD immunopathogenesis, particularly in patients with CD autoantibodies without intestinal damage [19]. The potential antigen specificity of these CD8 CTLs in CD intestinal epithelium was recently identified as A-gliadin 123–132 and further studies indicate that CD8 CTL and T-cell activation are similarly triggered by dietary gluten [20,21]. The CD8 + T-cells have αβ or γδ T-cell receptor and form a component of the intestinal intraepithelial lymphocytes (IELs) in addition to CD4 + T-cells (especially regulatory CD4 + T-cells and Th17 CD4 + T-cells) and B-cells. [21] Th17 cells and regulatory T-cells are usually present in the inverse proportions with a high preponderance of regulatory CD4 + T-cells. Inflammation in CD is associated with CD4 + T-cell polarization to a Th1 or Th17 phenotype resulting in tissue damage and destruction either directly or through the activation of autoreactive CD8 + T-cells [17]. Other IELs which may potentiate inflammatory changes in coeliac disease include mucosa-associated invariant T-cell, regulatory T-cells and NK T-cells which respond to non-peptide autoantigens [22,23].

1.2. Clinical presentation and aetiology

Understanding the CD immunopathogenesis is key to its diagnosis and the improvement of CD testing. CD has a variable clinical presentation and can be categorized as classic (intestinal), non-classic (extraintestinal), subclinical, refractory (RCD), and seronegative CD [1,24,41,42]. This can result in delayed diagnosis [43]. Patients with classic CD often present with intestinal symptoms, such as diarrhoea, decreased appetite, malabsorption, abdominal pain and distention, but may also have extraintestinal symptoms, such as iron-deficiency anaemia, arthritis, and dermatitis herpetiformis [41,44]. Subclinical CD is often only detected during the screening of susceptible individuals for CD because the symptoms

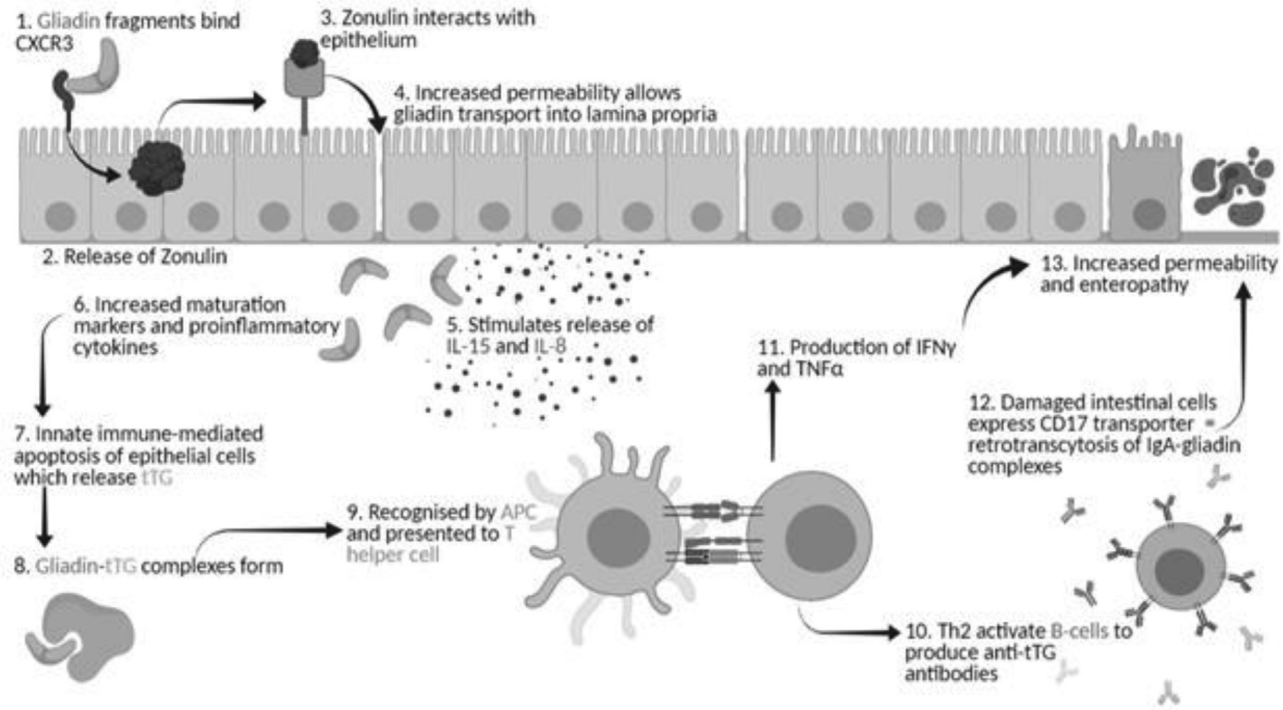

Fig. 1. The immunopathogenesis of CD is complex and multifactorial (Created with BioRender.com). CD is initiated by the binding of gliadin to CXCR3 (1) and the release of zonulin (2–3). This increases GIT permeability (4) with production of pro-inflammatory cytokines (5) and the presentation of gliadin to CD4 + T-cells by APCs (8–9) to initiate an adaptive immune response (10–12) further increases permeability and enteropathy (13).

Table 1
Commonly measured antibodies in CD[1].

ANTIBODY	*CURRENT USE*	*ACCURACY*
Anti-gliadin antibodies	Historical test now superseded [27–30]	
Anti-reticulin antibodies		
Anti-endomysial antibodies (EMA)	Routine	Low specificity High sensitivity [30,31]
Anti-tTG antibodies	tTG is the autoantigen of CD and a valuable diagnostic marker.[15,32–34] tTG-based serology tests have a Several comparisons with other antigens suggest that tTG is the superior diagnosis marker for CD.[35–37]	High sensitivity, accuracy, and efficiency.[1,34,35,38,39]
Anti-DGP antibodies	CD patients have T-cells specific for DGP. Antibodies against DGP are now a diagnostic marker.	High specificity High sensitivity [9,10,40]

[1] Selective IgA deficiency occurs in approximately 2–3% of CD patients and can render the IgA-based tests inaccurate. [24,25] It is recommended that total IgA is tested with every CD antibody test performed. In the presence of IgA deficiency, IgG antibodies against tTG and DGP are typically used.[26]

are not clinically significant [5]. There have been clinical cases of seronegative CD where serological markers are absent but there is severe malabsorption and villous atrophy [24,45]. Patients with RCD experience symptoms and villous atrophy after maintaining a gluten-free diet for a year or more [5]. The 2 subtypes of RCD are distinguished histologically by differences in IEL populations and have different prognoses [46].

Although gluten is the principal trigger for CD, several other environmental and genetic factors influence CD development [1,4,8,47,48]. These aspects of CD are not well understood but factors hypothesized to have a key role in gluten intolerance include intestinal infections, quantity and quality of gluten consumed, gut microbiota and infant diet [49,50].

1.3. Treatment and management

The only treatment for CD currently is a lifelong gluten-free diet (GFD) [1,5,8]. Although this has shown to relieve symptoms, decrease autoantibodies, and promote villous regrowth, a GFD can also cause poorer quality of life, mineral and vitamin deficiency, and increase cardiovascular risk [51,52]. Potential future therapeutics include dietary supplements to assist in digesting gluten, targeted therapies to induce tolerance, and inhibiting deamination of gluten by tTG [2]. Endopeptidases and zonulin inhibitors have been suggested as potential supplements to assist in gluten digestions and epithelial barrier restoration [2,8].

Misdiagnosis or late diagnosis of CD can increase morbidity and mortality because of complications. Common complications include iron deficiency and lowered bone mineral bone density leading to osteoporosis while lesson common complications include hyposplenism, refractory CD, intestinal lymphoma, small bowel adenocarcinoma and ulcerative jejunoileitis [53,54]. This provides additional motivation to establish biomarkers for CD as, currently, these complications require biopsies for diagnostic and prognostic information. This review addresses the current diagnostic algorithm for CD and examines potential future directions, specifically in the South African population.

2. Discussion

2.1. Serological testing

Serological testing for CD is accurate with sensitivities, specificities, and diagnostic accuracies greater than 90%, although testing is more accurate when individuals are tested whilst on a gluten-containing diet [55,56]. Autoantibody titers rapidly decline after GFD initiation in CD patients typically within a year. This is useful in monitoring dietary adherence and can be suggestive of GIT recovery and is associated with improved clinical symptoms [57]. Serological tests may have decreased accuracy for mucosal inflammation and recovery with RCD [58–60]. These challenges have resulted in the development of ancillary diagnostics like microbead-based technology, chemiluminescence, autoantibody profiling and regression tree analysis which aim to improve sensitivity and predict the requirements for biopsy [61–64].

2.2. Duodenal biopsies

Intestinal biopsy and histological examination confirms the diagnosis of CD in the context of positive serology [1,65]. Two to three biopsy samples are typically collected from the duodenal bulb and 4–6 biopsies from the distal duodenum using endoscopy [56,66]. The biopsies are graded histologically according to the degree of villous disruption [67].

There are different histological classification systems but the Marsh-Oberhüber histological classification method is the most widely used [65,68]. This system grades GIT disruption as Grade 1 (infiltration of IELs), Grade 2 (crypt hyperplasia), Grade 3 (partial (A), subtotal (B) or complete (C) villous atrophy) or Grade 4 (hypoplasia of the small bowel architecture) [1,65]. Duodenal biopsies are invasive but necessary confirmatory assays to establish the degree of disease and mucosal recovery following GFD. [18,69,70]

2.3. Supplemental testing

CD has a strong genetic component with a 10–15% familial recurrence and 75–80% concordance amongst monozygotic twins [8,71]. The HLA-DQA1*05:01-DQB1*02:01 haplotype, encoding the DQ2 molecule, is present in 90–95% of CD patients [72,73]. In the remaining approximately 5% of CD patients, the HLA-DQA1*03:01-DQB1*03:02 haplotype is expressed, encoding the DQ8 molecule. The remaining 5% of patients express at least one of the DQ2/DQ8 alleles [74]. This association provides a useful screening tool for CD through genotyping. The HLA DQ2/DQ8 genes, however, are required but not sufficient to cause CD limiting its utility as a standalone diagnostic test [8,75].

In the context of CD diagnosis, flow cytometry is mainly used to detect and count IELs to diagnose RCD when serology is unhelpful [76–78]. RCDII IEL characterization is typically indicated by the presence of clonal T-cells on flow cytometry [5,79]. These clonal T-cells typically express no surface CD3, CD4 or CD8 but have both cytoplasmic CD3 and CD103 but the lack of CD3 and CD8 with preservation of cytoplasmic CD3 and monoclonal rearrangement of T-cell receptor chain are the primary factors considered when diagnosing [5,77,80]. Although identifying IELs is primarily used to diagnose RCD, IELs can also be useful in diagnosing difficult cases of CD with inconclusive serology and histopathology [81,82].

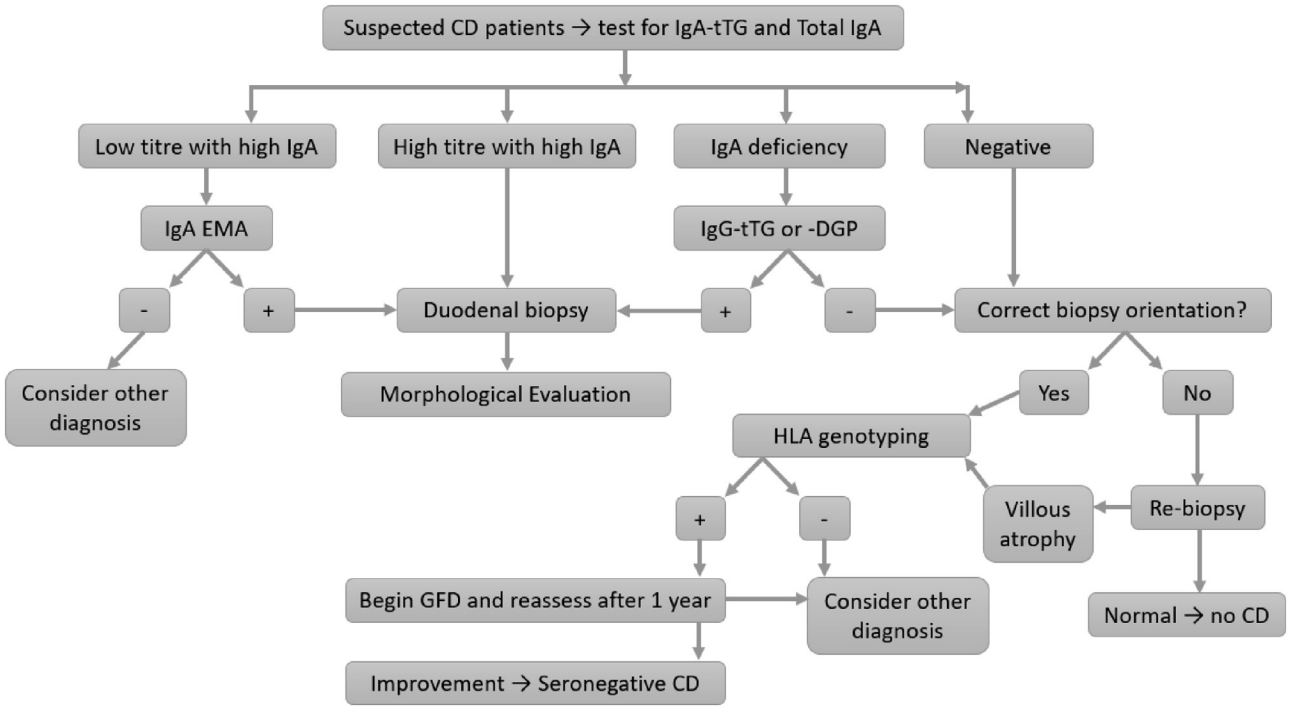

Fig. 2. The golden standard for the diagnosis of CD is serology with confirmatory histological features on duodenal biopsy. Currently the recommend serological test is IgA-tTG although this may be unreliable in patients with selective IgA deficiency. HLA typing may be used as a non-invasive screening test for CD.

Table 2
Potential diagnostic biomarkers identified in the last 10 years for CD.

Biomarker	Alterations seen in coeliac disease	Size	Population
Inflammatory Cytokines			
Serum levels IFN-γ, IL-6 and IL2	Increased	110	Iran [88]
		50	Australia [89]
Serum CX3CL1 levels	Increased	100	Spain [90]
Micro-RNA (mi-RNA)			
miRNA-146a and miRNA-155	Increased with high sensitivity and specificity	30	Chile [91]
miRNA-21 and −31	Increased	70	Egypt [92]
Faecal Components			
Volatile Organic Compounds (VOCs)	Different profiles	30	Netherlands [46]
Faecal Calprotectin	Increased	29	Germany [93]
Lipidomics			
Lipid and phospholipid profiles	Certain triacylglycerols are increased	233	Finland [94]
		256	Italy [95]
T-cells			
CD38 expression on gliadin-specific T-cells	Increased with gluten exposure	13	Norway [96]
Meta-analysis of TCRγδ + counting with flow cytometry[97]	High diagnostic accuracy	519	–
Oxidative Stress			
Levels of ROS and other biomarkers of oxidative stress	Correlates with the degree of villous atrophy	54	Italy [98]
Gene Expression			
Gene expression data from RT-PCR assays	Defined discriminant equations which could objectively and accurately classify duodenal biopsies into Marsh score categories	36	Australia [99]
Intestinal-derived Serum Proteins			
I-FABP[33]	Increased in untreated CD	20	Netherlands [100]
		141	Netherlands [101]
		108	Netherlands [102]
UBE2L3	Increased expression of (a ubiquitin ligase) predicts CD	9451	Spain [103]
Neo-epitopes of the tTG-DGP complex	Identify CD and mucosal healing	90	USA [104]
REG I α	Increased levels in CD and decreased with GFD	113	Spain [105]
Transcriptomics			
Transcriptomic signatures	Unique signature linked to increased cell proliferation, nuclear division, and cell cycle activity	121	Israel [106]

2.4. Diagnostic algorithms for CD

Global standards on CD diagnosis have been influenced mainly by European, British and North American guidelines which are widely published and have been summarized in Fig. 2 [1,5,56,83,84]. This diagnostic algorithm uses five antibodies for serology, IgA-tTG, total IgA, IgA-endomysial antibodies (EMA), IgA-DGP, and IgG-tTG or IgG-DGP (Fig. 2). Serology is followed up with a duodenal biopsy as final confirmation and HLA genotyping as an exclusion test when serology and biopsies are ambiguous. Canada, New Zealand and Australia follow similar guidelines [85–87]. The new European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) guidelines recommend no biopsy if tTG levels are 10 times greater than the normal limit and a second sample is EMA positive [83]. There is, however, little to no guidelines published in developing countries. The South African algorithm consists mainly of IgA-tTG and IgA-DGP serology with a follow-up biopsy. Diagnostic challenges in a resource-limited setting, such as South Africa, include loss of patient follow-ups and decreased availability of diagnostic testing.

2.5. Novel directions

Several new biomarkers are under development and could potentially improve the diagnostic, prognostic, and monitoring capabilities of CD testing (Table 2). The majority of these studies, however, were conducted outside of Africa. Key biomarkers include markers of inflammation (cytokines, reactive oxygen species (ROS) and genetic signatures of lymphocyte activation), biochemical markers of intestinal destruction (such as IFABP), and characterization of IEL populations. Recently, there has also been increased interest in the GIT microbiome because of its potential pathogenic role in CD.

Monitoring and prognostication are key in RCD because of the risk of malignant transformation [5,107]. Promising avenues of investigation include phospholipids profiling and mi-RNA detection [91,95]. Auricchio *et. al.* (2019) recently identified a serum phospholipid profile that distinguishes individuals who will develop CD before they present with symptoms or antibodies [95]. mRNA-146a, miRNA155, miRNA-21 and miRNA-125b have high specificity and sensitivity for active and inactive CD irrespective of treatment [91]. Investigations into these biomarkers in South African populations are needed as the novel research into CD diagnostics has occurred mainly in Europe and USA.

3. Conclusions

In conclusion, the expanding knowledge on CD pathology has enabled the construction of a complex diagnostic algorithm capable of accurately identifying individuals with CD, whereas the same cannot be said for many other autoimmune diseases. Regardless, several limitations need to be addressed through further investigations into the numerous avenues of research with the potential to improve the diagnosis, prognosis, and monitoring of patients with CD. In South Africa, the current diagnostic algorithm requires modification to better use resources and identify high-risk individuals.

Declaration of Competing Interest

The authors have no competing interests to declare.

None of the authors has any potential financial conflict of interest related to this manuscript.

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