

Serological Diagnosis of Celiac Disease –Moving Beyond the Tip of the Iceberg

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Abstract The majority of celiac disease affected patients are undiagnosed. Since the epidemiology and phenotype of CD is constantly changing towards latent, hyposymptomatic or asymptomatic behavior and since there is an increased risk of complications, early diagnosis and subsequent adherence to gluten-free diet is highly recommended. Multiple serological tests are on the market and the most frequently used test is IgA-tTG. This is not sensitive enough to be used alone, and combined tests enhance detection. Several combinations have been studied but not accurately compared to each other. The most frequently used combination is anti IgG-DGP with IgA-tTG, however, increasingly the new generation of anti neo-epitope DGP+tTG IgG+IgA is being used. At present, there is insufficient information to demonstrate that screening general populations definitely results in clinical benefit. However, asymptomatic individuals in high-prevalence groups should be screened.

Keywords: *celiac disease, diagnosis, serology, transglutaminase, CeliCheck, diaminated gliadin peptide*

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1. Introduction

Celiac disease (CD) is an autoimmune inflammatory disorder of the small intestine, triggered by the ingestion of prolamins contained in wheat, barley or rye, in genetically susceptible individuals. The prevalence of “suspected” celiac disease varies from 1 in 87 to 1 in 500 individuals in western countries. The majority of patients are undiagnosed since diagnosed cases of CD have a much lower prevalence being somewhere between 1 in 500 to 1 in 9000 individuals. In high risk populations, the average risk of CD can reach 5-10%.

There is an increased risk of complications such as hematological and gastrointestinal malignancies, osteoporosis/penia and other extraintestinal manifestations, decreased height, malnutrition and nutritional deficiencies, fertility impairment, stillbirth, dismaturity, psychosocial retardation, impairment of quality of life, increased mortality and additional autoimmune conditions, if left untreated. Thus early diagnosis and subsequent adherence to a gluten-free diet is highly recommended. The epidemiology and phenotype of CD are constantly changing. It has been shown that the classic intestinal clinical picture of malnutrition, chronic diarrhea and nutritional deficiencies are disappearing and extraintestinal presentations are emerging. Skin, endocrine, skeletal, hepatic, hematological, thrombophylic, gynecological, fertility, dental and behavioral abnormalities are often described. Nowadays, we are witnessing an epidemiological shift in the disease phenotype toward a more advanced age, and increased

prevalence of latent, hyposymptomatic or asymptomatic behavior [1,2]. All these changes make the diagnosis of the disease more difficult and the reliance on symptomatology more remote [3]. These are some of the reasons why serological screening and diagnosis of CD have achieved prime importance.

2. General or Case-Finding Screening

CD fulfills most of the major criteria for mass screening: it has a high prevalence, sensitive and specific biomarkers are available, it responds to a gluten-free diet, untreated it leads to morbidity/mortality, early detection is problematic and there are latent and early symptomatic stages. Despite this, mass screening for CD, as a public health intervention is controversial and data concerning the cost-effectiveness of case detection are sparse [4,5]. The main arguments against screening are: low compliance to a gluten-free diet in screen-detected patients, unacceptably low positive predictive values of current serological tests (given the pretest prevalence of less than 1%), poor understanding of the natural course of CD, conflicting mortality data with regard to undiagnosed CD and the above mentioned lack of data regarding cost-effectiveness. The general consensus is that there are insufficient data to justify mass screening of CD in the general population [4,5].

In contrast, screening targeted to high-prevalence groups may prove to have a favorable cost-benefit ratio, prevent or ameliorate associated autoimmune diseases and decrease complications including the risk of malignancy.

Arguments against screening high-prevalence groups include low adherence to a gluten-free diet and the lack of any improvement in quality of life [4]. The high-prevalence asymptomatic groups that are recommended for CD screening in children and adults are shown in Table 1.

Table 1. Asymptomatic, high risk groups who should be screened for celiac disease

1 st degree relatives.
Genetic syndromes: Down's, Ullrich-Turner, Williams-Beuren.
Autoimmune diseases: type 1 diabetes, autoimmune thyroiditis, autoimmune hepatitis, Addison's disease, multiple sclerosis, primary biliary cirrhosis, juvenile chronic arthritis.
IgA deficiency.

Adapted from references [4,5,6,7,8].

3. Diagnostic Autoantibodies in CD

The specificity and sensitivity of the serological markers is very wide-ranging from 29-100%. No single

marker is 100% sensitive and specific. It appears that the combination tests perform better, and are capable of detecting close to 100% of all celiac cases [4,5,9,10,14,17,18,23,24,25,26].

Despite the fact that the market for serological biomarkers is very dynamic and future studies can change the reported performance, looking at Table 1, at the present time, the following conclusions can be drawn: In children, the best single tests are IgA-tTG and EMA and the best combination test is Celicheck (antibodies against neo-epitope of the GDP-tTG complex). However, in adults, the best single tests is IgA-tTG and the best combination tests are IgA-tTG and EMA, and IgG-DGP/IgA-tTG. Our own experience [17,18,19] and that of many others [4,5,9,10,14,17,18,22,23,24,25,26] favors combination tests to screen for CD. The main ELISA kit candidates are anti tTG-IgA and anti-DGP IgG competing with the new Celicheck combination of IgA and IgG antibodies against the neo-epitope of the GDP-tTG complex, thus omitting screening for IgA deficiency [17,18,19,27,28].

Table 2. summarizes the sensitivity and specificity of the current serological biomarkers in CD patients

Serological test	Sensitivity Mean (range),%	Specificity Mean (range),%	Sensitivity Mean (range),%	Specificity Mean (range)%	References
Single tests	In children	In children	In adults	In adults	
tTG IgA	≥90 (74-100)	≥90 (78-100)	77-100 93(91.2-94.5)	91-100 96.5(95.2-97.5)	[8,9] [10]
tTG IgG	57-95	94-100	29-100	84-100	[11,12,13]
EMA- IgA	≥90 (75-96)	98 (91-100)	61-100	80-100	[8,9,14]
DGP- IgG	(80-98.6)	(86.0-96.9)	56-94 87.8(85.6-89.9)	90-99.3 94.1(92.5-95.5)	[9,11,12] [10]
DGP- IgA	>80(80.7-95.1)	>90(86.3-93.1)	84.3	79.8	[9,15]
Combination tests					
IgA-tTG and EMA			98-100	98-100	[8]
CeliCheck=Neo-epitope tTG complexed to DGP IgA+G and IgA-tTG	(98-100)	(93-96.2)	94-96	92	[16,17,18,19]
IgA+G-tTG/DGP	100	89-97	100	96-97	[15,20,21,22]
IgGDGP/IgAtTG			100	97.5	[21]
IgADGP/IgAtTG			100	92.6	[21]

Formation of the tTG-DGP complex is known to involve epitope spreading from gliadin to tTG [28]. The antibodies against neo-epitopes of the tTG-DGP complex provide a new screening and diagnostic test in CD. Multiple studies have exhibited diagnostic sensitivities of 95% and specificities of 97% or more, when compared with those of traditional antibody assays [16,29]. The neo-epitope tTG/DGP is able to drive the development of 3 different autoantibodies: against DGPs, against tTG and against newly formed epitopes derived from the cross-linkage between the enzyme and the substrate. It was suggested that these neo-epitope directed antibodies appear early during the development of CD, preceding the formation of anti DGP and anti tTG, through a mechanism of epitope spreading, imitating the appearance of autoantibodies in SLE. It is foreseeable that the autoantibodies generated against the neo-epitope complex may represent the best means for screening populations and for diagnosing high-risk groups for identification of the silent or latent patients. In fact, several studies have shown the superiority of screening for CD using the tTG/DGPs complex strategy in the general population

[29,30] or in groups of at-risk subjects [17,18,19,31,32,33]. Most recently, neo-epitope DGP/tTG autoantibodies were shown to present a new and sensitive serological marker of dermatitis herpetiformis, a disease closely related to CD [34].

4. Summary

Since only the tip of the CD iceberg is above the waterline and the much larger portion of the CD iceberg remains undetected underwater, it can be expected that the prevalence of the disease will continue to increase and that the presenting symptoms of the disease will continue to change towards a/hyposymptomatic ones, supporting the need to screen and diagnose the disease. At present, there is insufficient information to demonstrate that screening the general population definitely results in clinical benefit. However, asymptomatic individuals in high-prevalence groups should be screened. Multiple serological tests exist on the market and the most frequently used one is IgA-tTG. This is not sensitive enough to be used alone, and is better combined with other tests. Several combinations

have been studied but not accurately compared with each other. The most frequently used is anti IgG-DGP and IgA-tTG, however, the new generation anti neo-epitope DGP+tTG IgG+IgA is increasingly being used.

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