

Speeding up coeliac disease diagnosis in cardiological settings

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Abstract

Introduction: High prevalence of coeliac disease (CD) has been reported among patients with idiopathic dilated cardiomyopathy (DCM). We evaluated the feasibility and diagnostic accuracy of screening for CD by rapid test of anti-transglutaminase antibodies in the cardiology outpatients' clinic.

Material and methods: We screened the blood samples of 104 patients with DCM, 44 of their first-degree relatives, 63 diseased controls and 101 healthy controls for the presence of anti-transglutaminase antibodies in a drop of whole blood using a rapid assay. This test was compared to the enzyme-linked immunosorbent assay and the anti-endomysium antibody test.

Results: Our rapid test was positive in three (2.9%) DCM patients, in one (2%) relative and in one (1%) healthy control. These subjects were positive at both control assays. Two DCM patients had iron-deficient anaemia. The healthy relative was asymptomatic, while the healthy control experienced extreme asthenia. The relative refused intestinal biopsy, while the others showed histological evidence of CD. During the gluten-free diet, the patient with the worst left ventricular ejection fraction (LVEF) underwent heart transplant, and LVEF values improved in the other two. Anaemia and tiredness resolved in all patients.

Conclusion: Early detection of CD in a cardiological setting allows prompt treatment with a gluten-free diet of gluten-dependent complaints with potential benefits for the course of DCM.

Key words: anti-transglutaminase antibodies, coeliac disease, dilative cardiomyopathy, screening.

Introduction

Coeliac disease (CD) is a genetically determined lifelong intolerance to gluten from dietary cereals, with a 1% prevalence among the general population, but a much higher one (8-20%) among at-risk populations (first-degree relatives of coeliacs, and patients with autoimmune disorders) [1, 2]. In CD, regular ingestion of wheat, rye and barley induces T-cell mediated inflammation in the gut and an autoimmune response to self-proteins, mainly tissue type-2-transglutaminase, with the production of anti-tissue-transglutaminase antibodies (anti-tTG) [3].

In general, the *classic* clinical presentation is seen in subjects with gastroenterological complaints comprising chronic diarrhoea, abdominal

distention and pain, and sometimes malabsorption. In the now more common *atypical* presentations of the disease, gastrointestinal symptoms may be absent or less pronounced, and extra-intestinal features may be present such as anaemia, osteoporosis, short stature, infertility, neurological and cardiological problems, and associated autoimmune diseases [2]. Because atypical presentations are increasingly found to predominate, CD is now considered a multi-system disorder involving all medical specialities for its diagnosis. Early diagnosis and treatment with a gluten-free diet (GFD) significantly reduces mortality and prevalence of CD-associated disorders [4]. Although definite diagnosis of CD is still based on certain characteristic histological changes in the jejunal intestine, serological tests for CD screening based on detection of anti-tTG are cheaper and less invasive, with excellent sensitivity (98%) and specificity (95%) [5]. Given the high prevalence of the disease, the implications of missing diagnosis (or late recognition), and the involvement of all the medical specialities, simple blood drop based immunoassays have been developed and validated for measuring anti-tTG, in order to speed up CD diagnosis in gastroenterology settings [6] and screen the general population [7].

Although the association between CD and heart diseases is not clearly established [8, 9], several reports indicate a higher CD prevalence among patients with idiopathic dilated cardiomyopathy (DCM) and myocarditis [10-12].

In a very recent systematic revision a higher prevalence of CD was confirmed among patients with cardiomyopathy; in this subgroup of patients there was also reported an improvement of cardiac performance after introduction of the GFD. Based on these data the authors suggested to consider routine CD screening in all patients suffering from cardiomyopathy [13].

Here, we explore the feasibility of screening patients with DCM and their first-degree relatives for CD using a rapid anti-tTG assay, to be performed by a nurse or cardiologist in the course of daily practice in a third-level referral clinic for cardiology, and we compared the rapid test results with those obtained by using a well-established immunoenzymatic assay.

Material and methods

Subjects

From April 2007 to February 2008, 312 subjects underwent screening: 104 adults with DCM (59 males, 45 females, median age 52 years, range 28-61), 44 first-degree relatives of these patients (27 males, 17 females, median age 44 years, range 18-55), 63 diseased controls (43 males, 20 females,

median age 59 years, 39-72) and 101 apparently healthy nurses and residents working in cardiology or paediatric departments (60 males, 41 females, median age 40 years, range 27-50). The diagnosis of DCM was based on WHO criteria at the Cardiovascular Department, Azienda Ospedaliera-Universitaria of Trieste [14].

Diagnosis of DCM was based on clinical evaluation, 12-lead ECG, echocardiography (global and regional kinetic evaluation, ventricular size, valvular regurgitations, haemodynamic evaluation), Holter monitoring, exercise test and, when indicated, coronary angiography, invasive haemodynamic evaluation, and endomyocardial biopsy. Relatives who agreed to be screened underwent clinical evaluation, 12-lead ECG and echocardiography (global and regional myocardial function, ventricular size, valvular regurgitations, and haemodynamic evaluation). Diseased controls were suffering from acute coronary syndrome ($n = 42$), acute pericarditis ($n = 3$), valvular diseases ($n = 8$), and severe ischaemic heart failure ($n = 10$).

Laboratory testing for coeliac disease

The rapid test for detecting IgA anti-tTG, based on a whole blood drop, was used as previously described [6] following the manufacturer's instructions (Eu-tTG Quick, Eurospital, Italy). Anti-tTG were measured by nurses using sticks incubated for 5 minutes in a solution containing 10 μ l of whole blood from a finger prick. Positive results appear as varying shades of grey bands on the sticks (Figure 1). The shades were interpreted by two operators blind to subjects' histories and laboratory findings.

At the same time, the nurses took an additional 80 μ l of finger prick blood which was centrifuged: the supernatant was tested for both IgA and IgG anti-tTG using an ELISA assay (Eu-tTG IgG, human IgA, Eurospital, Italy), following the manufacturer's instructions and for IgA anti-endomysium antibodies (AEA), by using an immunofluorescence assay on human umbilical cord cryosections as previously described [15]. These assays were performed 5-10 days after the rapid test by two operators blind to the rapid test results. Subjects testing negative for anti-tTG antibodies were categorized as not having CD.

The DQA1*05/ DQB1*02 and DQA1*03/DQB1*0302 alleles, encoding the DQ2 and DQ8 heterodimers respectively, were detected in subjects with positive antibody tests by allele-specific PCR, using Dynal SSP Kits (Dynal).

Small bowel biopsies

Patients positive for anti-tTG or AEA or for both were advised to undergo small intestine biopsy,



Figure 1. Positive and negative results for anti-transglutaminase antibodies using the rapid immunoassay. The first band (head-arrow) must always appear as a technical control, the second band (arrow) represents the positive result of the test. No second band was detected in the first strip incubated with a blood sample that tested negative for anti-transglutaminase antibodies. Different shades of grey bands identified coeliac patients in the second and third strip

analysed according to Oberhuber's classification [16]. Patients whose results were histologically positive were considered to have CD and were started on GFD. The present study was approved by the local independent Ethical Committee.

Results

Three patients with DCM (2 males, 1 females, 3/104, 2.9%), 1/44 (2.2%) relatives, who was the daughter of a DCM patient, and one nurse among the 101 (1%) controls tested positive to the rapid test and to both the anti-tTG ELISA and AEA assays. All 5 carried the HLA DQ2 heterodimer: four agreed to undergo intestinal biopsy which showed total villous flattening (type 3c) in all cases. The DCM patient's daughter refused intestinal biopsy, but due to the extremely high positive predictive value (100%) of co-presence of AEA, anti-tTG and CD-related HLA, this subject was considered as having CD. The remaining 307 subjects (101 DCM patients; 63 sick controls; 100 healthy controls; 43 first-degree relatives) who tested negative to the rapid test were confirmed as negative by both anti-tTG and AEA assays.

We did not find differences in rapid test results compared to those obtained by ELISA assay.

At the time of CD diagnosis one DCM patient presented with severe left ventricular dysfunction (LVEF 20%), one DCM patient complained of recurrent abdominal pain and iron-deficiency anaemia (Hb 9g/dl; mean corpuscular volume 68 fl; ferritin serum level 6 ng/ml), and one DCM patient presented with iron-deficiency anaemia (Hb 11 g/dl; mean corpuscular volume 71 fl; ferritin serum level 11 ng/ml) and severe asthenia (Table I).

Twelve out of 101 patients affected by DCM without CD presented with anaemia (mean value 9.5 ± 2 g/dl); in these patients normal mean corpuscular volume values (mean value 82 ± 6 fl) and serum ferritin concentration (mean value 212 ± 37 ng/ml) were found. The healthy relative was asymptomatic and refused both the diagnosis of CD and diet therapy. The healthy control experienced severe tiredness without anaemia.

Six months after CD diagnosis, the DCM patient with the worst LVEF value (20%) underwent heart transplant. Over 16 months of GFD, the LVEF values improved in the other two DCM patients (respectively from 38% to 47% and from 46% to 60%) and the repetitive ventricular arrhythmias detected in one were no longer present in serial Holter recordings. The GFD improved the anaemia in two patients with DCM and tiredness resolved in the third DCM patient and in the healthy control.

Discussion

This study shows that an easy, reliable test for detecting IgA anti-tTG antibodies makes quick diagnosis of CD possible in a cardiological setting. This rapid assay is just as accurate as specialized laboratory testing, has extremely high sensitivity and specificity values, takes fifteen minutes to obtain a result and fits in comfortably with the nurse's normal working schedule. Being able to administer such a simple test in the course of routine clinical follow-up examinations helps in early detection of CD and, in the case of positive results, in recognizing CD as the causative agent of otherwise not fully understood clinical symptoms such as tiredness and anaemia. Furthermore, this test could be used in a larger study population in order to establish the real prevalence of CD in patients suffering from heart diseases. In adulthood, complaints of a lifetime of tiredness and anaemia are common and they represent the most frequent symptoms found in delayed CD diagnosis. In a recent Canadian coeliac survey [17], 40% of adult coeliacs had consulted two or more physicians (including a haematologist) for untreatable anaemia prior to CD diagnosis, and the mean delay in CD diagnosis after onset of symptoms was 11 years. Interestingly, no patients demonstrated DCM clinical signs among this cohort; but this might be due to the fact that only 50% of the coeliacs actively participated in the clinical survey.

Table I. Clinical characteristics of patients with dilated cardiomyopathy and coeliac disease before and after the gluten-free diet

	Case 1 Female	Case 2 Male	Case 3 Male
Disease presentation	Recurrent perimyocarditis (DCM post-myocarditis)	Single episode of perimyocarditis with HF (DCM post-myocarditis)	DCM
Family history of myocardial diseases	No	No	No
Age at DCM diagnosis	27 years	30 years	36 years
Age at CD diagnosis	37	35	37
Associated complaints	Anaemia (11 g%), severe asthenia	Anaemia (9 g%), recurrent abdominal pain	Chronic fatigue
CV therapeutic changes after CD diagnosis	No	No	Added bisoprolol
Clinical and laboratory changes during the gluten-free diet			
Follow-up [months]	16	6	16
NYHA class	II-III → I	IV → heart transplantation 6 months after CD diagnosis	II → I
LVEF [%]	38 → 47	20 → transplanted	46 → 60
LVEDd [cm]	5.8 → 5.6	6.3 → transplanted	6.3 → 6.1
Number of ventricular extrasystoles	1685 → 1083	Not available	7953 → 921
Repetitive ventricular events (couples, triplets)	No	Not available	Yes → No
Evolution of symptoms	Severe asthenia → no symptoms	Recurrent abdominal pain, chronic diarrhoea → no intestinal symptoms	Chronic fatigue → no symptoms
Hb values [g/dl]	11 → 13	9 → 14	14 → 14
IgA -IgG anti-tTG	+ → -	+ → -	+ → -

DCM – dilated cardiomyopathy, CD – coeliac disease, HF – heart failure, CV – cardiovascular, NYHA – New York Heart Association, LVEF – left ventricular ejection fraction (normal value > 50%), LVEDd – left ventricular end diastolic dimension, Hb – haemoglobin, anti-tTG – anti-tissue transglutaminase antibodies

Also in our own patients, anaemia and chronic fatigue had a negative effect on clinical management, which resolved promptly thanks to GFD, with a great improvement in their quality of life. As previously reported [11, 12], iron-deficiency anaemia could be a useful clinical marker for arousing suspicion of CD in patients with cardiomyopathy, and to use the rapid test described above. Interestingly, those patients without CD, but presenting anaemia, did not show laboratory findings indicating iron-dependent anaemia.

Diagnosis of CD in a DCM patient might also help to treat the cardiac dysfunction, through compliance with GFD, as reported by clinical anecdotal experience [13]. However, further studies are needed to confirm the hypothesis of a positive effect of GFD on the course of DCM. In other autoimmune diseases associated with CD, such as autoimmune hepatitis [18], alopecia [19],

gluten-dependent ataxia [20] and dermatitis herpetiformis [21], GFD works as a specific therapeutic agent.

This quick and simple test performed on patients with DCM in a day-clinic setting is both effective and cheap (around € 6 per test vs. € 16 for IgA-IgG ELISA assay), and could reduce delays in CD diagnosis and allow prompt treatment with GFD of gluten-dependent symptoms.

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