



INTESTINAL ANTITRANSGLUTAMINASE ANTIBODIES TO DISCOVER GENETIC GLUTEN INTOLERANCE

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Abstract — Genetic gluten intolerance is a multifactorial condition characterized by the intestinal synthesis of antitransglutaminase antibodies (anti-tTG) which might represent an early stage of this intolerance in absence of both intestinal damage and serum anti-tTG. The double immunofluorescence staining (IF) is able to point out these anti-tTG antibodies directly on intestinal biopsy. Here we describe a prospective study in which patients were analysed for genetic predisposition (HLA DQ2-DQ8) and serum anti-tTG and were monitored for clinical conditions and serum anti-tTG concentration during gluten free diet (GFD) or gluten containing diet (GCD). Our results demonstrate that the measurement of intestinal anti-tTG is a useful screening procedure to identify patients with genetic predisposition not fulfilling the current diagnostic criteria.

Index Terms — TRANS2CARE, genetic gluten intolerance, antitransglutaminase antibodies, immunofluorescence

1 BACKGROUND

The current diagnostic criteria for genetic gluten intolerance require intestinal mucosal villous atrophy and the presence of serum anti-tissue transglutaminase (anti-tTG) antibodies. Anti-tTG antibodies are synthesised by specific B lymphocytes in the small bowel mucosa at an early stage of gluten intolerance even in absence of both intestinal damage and serum anti-tTG.

2 OBJECTIVES

Our objectives are:

to evaluate by double immunofluorescence staining (IF) the intestinal anti-tTG in patients undergoing to gastrointestinal endoscopy

to correlate IF results with clinical, serological, histological and genetic markers

3 APPROACH & METHODS

General approach

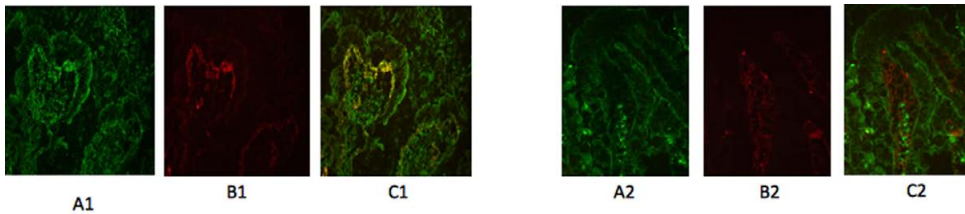
This is a prospective study. Patients were analysed for clinical (anaemia, diarrhea, abdominal pain), genetic (HLA DQ2/8), serological (serum anti-tTG), histological (villous atrophy) and intestinal markers. Intestinal markers are intestinal antitransglutaminase antibodies searched with double immunofluorescence staining. Patients were monitored for clinical conditions, serum anti-tTG concentrations during gluten free diet (GFD) or gluten containing diet (GCD).

Methods

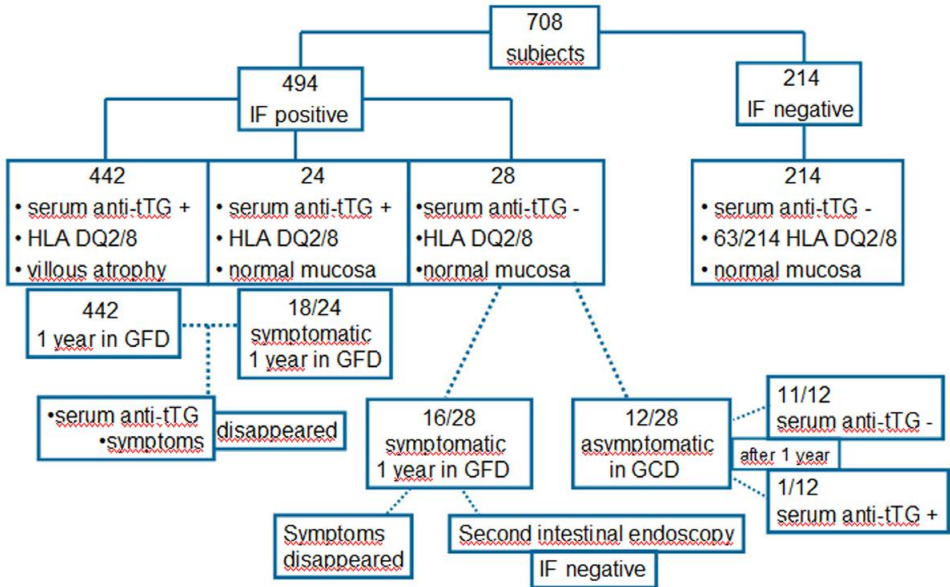
The double immunofluorescence staining technology is applied on intestinal crio-sections in order to point out the IgA antibodies against tissue transglutaminase (tTG) by using labelled antibodies. IgA are labelled with fluorescein (green fluorescent signal) and tTG with rhodamine (red fluorescent signal). Through the fluorescent microscope examination, two pictures are taken from the same section: one representing IgA distribution (figures A1 and A2) and the other one tTG distribution (figures B1 and B2). The overlap of these images, by using an appropriate software, results in a third picture where the IgA against tTG are pointed out with a yellow signal obtained from the localization of green IgA signal and red tTG signal at the same area (figure C1). If IgA against tTG are not synthesised, no yellow signal is raised (figure C2).

4 RESULTS

Intestinal anti-tTG identified 24/708 (3%) and 28/708 (3.5%) subjects with normal mucosa and with positive and negative serologic markers, respectively. Half of them (18/24 and 16/28) clinically improved in GFD.



5 POTENTIAL NEW PRODUCTS & SERVICES



Since these autoantibodies are produced at the intestinal level at an early stage, when they are not serologically detectable and the small intestinal mucosa is morphologically normal, this approach could be very useful for early diagnosis. This technology is already a service described on the IRCCS Burlo Garofolo website as a new test to make diagnosis of genetic gluten intolerance at an early stage. University Medical Center Maribor already benefits from this service.

6 CURRENT COLLABORATIONS

6.1 With other researchers

Morena Silvestrini, Ca'Foscari Venice (PP7, Trans2Care)

6.2 With hospitals

University Medical Center Maribor

7 CONTACT OR COLLABORATIONS NEEDED

Our results indicate that the double immunofluorescence staining, identifying intestinal anti-tTG antibodies, is able to discover new clinical conditions of genetic gluten intolerance. In order to extend the application of this technology in diagnostic field, it's necessary to start collaborations with many other clinical units (as done with University Medical Center Maribor) and companies interested in translational research.

8 COMMUNICATION TOOLS

- Radiol Oncol. 2013 May 21;47(2):128-37
- Curr Pharm Des. 2012;18(35):5753-8
- FASEB J. 2011 Jan;25(1):144-58

9 FUNDS NEEDED

9.1 For applied research (solutions for real-world problems): 25.000 €

9.2 For pilot & demonstrator activities (to develop a prototype): 30.000 €

10 CONCLUSION

The measurement of intestinal anti-tTG is a useful screening procedure to identify patients with genetic predisposition (HLA DQ2/8) not fulfilling the current diagnostic criteria that promptly respond to GFD.

Acknowledgement

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