TISSUE BIOMARKERS FOR THE DIAGNOSIS OF CD

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Background

The diagnosis of celiac disease (CD) requires the detection of villous atrophy and crypt hyperplasia in small-bowel biopsy, in presence of high serum titers of specific-CD autoantibodies in genetically predisposed subjects. It is now accepted that, from a histological point of view, CD represents a wide spectrum of histological picture as it goes from the typical severe lesions of duodenal mucosa with villous atrophy, hyperplasia of the crypts, increased infiltration of the intraepithelial lymphocytes, to forms characterized by minor degrees of enteropathy. Therefore, the correct orientation of duodenal specimen during cutting is of great importance in order to obtain correct quantitative histological measurements, such as the villous height crypt depth ratio (VH: CrD) and intraepithelial lymphocyte (IEL) density. Furthermore, CD encompasses a large spectrum of clinical, and serological manifestations that range from serious symptomatic forms to completely asymptomatic forms. In clinical practice, it is increasingly more common to find patients who have serum positivity for CD-related antibodies but whose duodenal mucosa shows a normal histological appearance, the so-called potential CD. For these individuals, the diagnosis is difficult because even though several markers of gluten sensitivity have been proposed, such as the increased number of $\gamma\delta$+IELs or the count of intraepithelial lymphocytes at the villous tip, none of them is so specific as to identify with certainty those patients in whom clear intestinal mucosal damage will develop and for whom a gluten-free diet is necessary. A new biomarker has been identify in the last 20 years. As evidenced anti-tissue transglutaminase2 (anti-TG2) are primarily produced at an intestinal level by TG2-specific plasma cells and deposited in the intestine forming mucosal deposits. TG2 specific IgA deposition precedes both the formation of small intestinal damage and the detection in the circulation of the same antibodies. The presence of mucosal deposits of anti-TG2 antibodies seems to be a predictive marker of future evolution to villous atrophy in potential CD.

Main achievements

Our principal aim is to search reliable markers of gluten-dependent enteropathy. As known, among the immunohistochemical markers, one of the best predictors of CD diagnosis is the increase in γδ+ IELs. We showed that, firstly, the γδ+/CD3+ ratio is considered a better marker of gluten sensitivity than CD3+ and γδ+ values considered singly, then an improved immunohistochemical approach, based on the count of CD3+, γδ+ IELs, and lamina propria CD25+cells, may be of help in identifying subjects with gluten-dependent enteropathy among those with normal mucosal architecture.

Furthermore, our group validated the detection of anti-TG2 mucosal deposits as useful diagnostic test for the diagnosis of childhood CD, showing a sensitivity of 96% and a specificity of 88%. We tested the presence of intestinal deposits of autoantibodies in CD patients in all stages of disease: in the active phase, in remission and in our cohort of potential CD patients. In particular, mucosal deposits were detected in 68% of potential CD, it was considered a predictive marker of future evolution to villous atrophy, as it was seen more often in those potential patients that progress to overt CD than in those remaining in early phase of disease. Moreover we proved that intestinal anti-TG2 antibody production does not show absolute specificity for CD. It is seen more often in association with inflamed mucosa in patients with diagnosis other than CD. Finally, anti-TG2 deposits were seen in the majority of T1D patients and in relatives of CD patients, two groups at risk to develop CD.

Another assay, in use in our laboratory, reveals intestinal production of anti-TG2: the measurement of these autoantibodies by ELISA test in supernatants from 24h of small intestinal cultures. We proved this assay has higher sensitivity and specificity (97·5 and 92·3%, respectively) than the detection of mucosal deposits (77·5 and 80·0%, respectively) to reveal intestinal production of specific CD autoantibodies. The measurement of intestinal anti-TG2 antibodies may prove useful in clinical practice to predict evolution towards mucosal atrophy in potential coeliac patients and identify patients with gluten sensitivity. Finally, we showed that intestinal anti-TG2 antibodies titers correlated positively with the degree of mucosal damage and inversely with the duration of GFD.

Future perspectives

We aim to investigate intestinal production of anti-TG2 antibodies, as this phenomenon could be suggestive of a very early condition of gluten reactivity. The subjects initially producing anti-TG2 antibodies only at an intestinal level could become serum positive later and eventually develop small intestinal mucosal damage. To improve the

detection of subjects with abnormal response to gluten (and lack in the serum of CD associated autoantibodies) the data about IgA anti-TG2 intestinal deposits will be integrated with other mucosal markers of "gluten sensitivity" such as increased density of gammadelta intraepithelial T cells.

Publications

1. *Borrelli M, Maglio M, Korponay-Szabó IR, Vass V, Mearin ML, Meijer C, Niv-Drori H, Ribes-Koninckx C, Roca M, Shamir R, Troncone R, Auricchio R. Intestinal anti-transglutaminase 2 immunoglobulin A deposits in children at risk for coeliac disease (CD): data from the PreventCD study. Clin Exp Immunol. 2018; 191:311-317. doi:10.1111/cei.13078.*
2. *Maglio M, Tosco A, Auricchio R, Paparo F, Colicchio B, Miele E, Rapacciuolo L, Troncone R. Intestinal deposits of anti-tissue transglutaminase IgA in childhood celiac disease Dig Liver Dis. 2011; 43:604-8. doi:10.1016/j.dld.2011.01.015.*
3. *Maglio M, Florian F, Vecchiet M, Auricchio R, Paparo F, Spadaro R, Zanzi D, Rapacciuolo L, Franzese A, Sblattero D, Marzari R, Troncone R. Majority of children with type 1 diabetes produce and deposit anti-tissue transglutaminase antibodies in the small intestine. Diabetes 2009; 58:1578-84.*
4. *Tosco A, Maglio M, Paparo F, Rapacciuolo L, Sannino A, Miele E, Barone MV, Auricchio R, Troncone R. Immunoglobulin A anti-tissue transglutaminase antibody deposits in the small intestinal mucosa of children with no villous atrophy. J Pediatr Gastroenterol Nutr 2008; 47:293-8.*
5. *Tosco A, Maglio M, Paparo F, Greco L, Troncone R, Auricchio R. Discriminant score for celiac disease based on immunohistochemical analysis of duodenal biopsies. J Pediatr Gastroenterol Nutr. 2015; 60:621-5. doi:10.1097/MPG.0000000000000675.*
6. *Tosco A, Aitoro R, Auricchio R, Ponticelli D, Miele E, Paparo F, Greco L, Troncone R, Maglio M. Intestinal anti-tissue transglutaminase antibodies in potential coeliac disease. Clin Exp Immunol. 2013; 171:69-75. doi:10.1111/j.1365-2249.2012.04673.x.*
7. *Tosco A, Salvati MV, Auricchio R, Maglio M, Borrelli M, Coruzzo A, Paparo F, Boffardi M, Esposito A, D'Adamo G, Malamisura B, Greco L, Troncone R. Natural history of potential celiac disease in children. Clin Gastroenterol Hepatol 2010; 9:320-25.*
8. *Tosco A, Auricchio R, Aitoro R, Ponticelli D, Primario M, Miele E, Rotondi Aufiero V, Discepolo V, Greco L, Troncone R, Maglio M. Intestinal titres of anti-tissue transglutaminase 2 antibodies correlate positively with mucosal damage degree and inversely with gluten-free diet duration in coeliac disease. Clin Exp Immunol. 2014; 177:611-7. doi:10.1111/cei.12366.*
9. *Auricchio R, Mandile R, Del Vecchio MR, Scapaticci S, Galatola M, Maglio M, Discepolo V, Miele E, Cielo D, Troncone R, Greco L. Progression of Celiac Disease in Children With Antibodies Against Tissue Transglutaminase and Normal Duodenal Architecture. Gastroenterology. 2019; pii: S0016-5085:35671-9. doi:10.1053/j.gastro.2019.04.004*
10. *Maglio M, Ziberna F, Aitoro R, Discepolo V, Lania G, Bassi V, Miele E, Not T, Troncone R, Auricchio R. Intestinal Production of Anti-Tissue Transglutaminase 2 Antibodies in Patients with Diagnosis Other Than Celiac Disease. Nutrients. 2017; 9(10). pii: E1050. doi:10.3390/nu9101050.*