Outside of Normal Limits:
False Positive/Negative Anti TG2 Autoantibodies

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Abstract   Detection of IgA-transglutaminase2 (TG2) autoantibodies has become the test of choice for the diagnosis and monitoring of celiac disease (CD), and is recommended as the serological 'gold standard' by most of the CD societies. Despite its wide acceptance and reasonable performance, several aspects are problematic and disputed. The normal range levels between positivity and negativity are modified, manufacturers' cut-off levels are extremely variable, far from matching in house determinations, insufficient standardization, inadequate reference protocols and no reliable quality assessment of the ELISA antibody (AB) kits for CD diagnosis and follow-up of dietary adherence. Numerous limitations exist in its detection, diagnosis, and follow-up capacities, resulting in frequent clinical circumstances when professionals encounter false positive and negative situations. The present review updates and discusses these facets and aims to extend our knowledge and help CD associated professionals to use the IgA-TG2 antibodies appropriately. We hope that the content of the review will stimulate the scientific community to explore and better delineate the role and functions of the AB and the industrial manufacturers to improve clinical performance of their diagnostic tests.

Keywords: tissue transglutaminase, TG2, false negative, false positive, diagnosis, antibodies

1. Introduction

The diagnosis of CD relies upon the concordance of clinical, histological, serological and genetic features making it often a medical challenge. In reality, facing the bed and not the bench, not all the above features exist to confirm the diagnosis. In the past, an elevated AB level was regarded as the main indication for a subsequent biopsy. With the advance of serological biomarkers and amelioration of their diagnostic reliability, they became essential to achieve cost-effective case-finding in CD [1,2]. Accepted assay sensitivities are above 74 and 81 and specificities above 99 and 97, for anti endomysial (EmA) and anti tissue transglutaminase (tTg), antibodies, respectively [3]. Good assay performance and reliability, were the basis for omitting an intestinal biopsy, in certain circumstances in pediatric CD, as per the revised ESPGHAN diagnostic criteria [4]. However, the CD committee recommended IgA-tTg and IgA-EmA but failed to mention the other CD associated autoantibodies (anti IgA/IgG deamidated gliadin peptide, anti IgA/IgG neo-epitope tTg) in their diagnostic flow chart [5,6,7]. Very recently, neo-epitope tTg AB test were found to outperform tTg AB tests [8]. The newer serological markers of CD, including anti TG2, are more accurate than many other autoantibodies currently used in autoimmune diseases, however, they are gluten consumption dependent.

Due to the pivotal importance of anti tTg AB in screening and diagnosis of CD, the present review will concentrate on false + and false – detection of the serological "gold standard" anti IgA-tTg autoantibody. The aim being to increase the awareness of the professionals for the frequency and the significance of the titers that are above or below the normal ranges of IgA-tTg, in face of celiac versus non-celiac disease, respectively.

2. Normal Limits and Limitations of ELISA Anti TG2 ABs

Many clinically used serological ELISA kits of anti TG2 ABs are available, and they have a wide range of different cut-off levels. This is the main reason why one cannot define their precise normal limits. It is easier to decide on the relative lower normal limit, which is IgA-Tg2<1 ULN and intermediate relative levels between 1ULN and above [9]. However, the upper cut-off levels provided by the manufacturers for their IgA-TG2 ELISA determinations are so variable, making back-to-back comparisons, over time and across populations quite difficult. The situation is further complicated by the local, in house laboratory determinations of the upper limit cut-off, frequently different from the manufacturers' ones. It goes without saying that these different cut-off levels impact the performance of the test, namely sensitivity,

specificity and positive and negative predictive values. Literature surveys on the sensitivity and specificity of IgA-TG2 is marked by extensive heterogeneity in the kit's performance, depending on both the commercial assay variant used and the cut-offs provided by the supplier. The significant range of test accuracies makes selection of their mean performances very arbitrary. In fact, several studies investigated specifically the variation in test results of different manufactures' kits on the same individual sera and showed a wide range of variability [10,11,12]. Manipulating the cut-offs or using a combination test, combining a more specific test with a more sensitive one, affects dramatically the results. As an example, Barak et al, reported that the sensitivity of the neo-epitope TG2 is higher than that of IgA-TG2 at all levels while the specificity is somewhat lower. As expected, the sensitivities of the combined results of >10,>5, or >2 UNL of either neo-epitope or IgA-TG2 were improved in comparison to each test alone [12]. The manipulations of the cut-offs and the combination of the tests, might greatly affect the recent diagnostic flow chart of ESPGHAN [4] concerning the necessity for HLA determination and the need to perform a small bowel biopsy [9,12].

It is well known that PPV and NPV depend strongly on the prevalence (pre-test probability). The ESPGHAN X10ULN cut-off suggestion was derived from essentially 3 studies [13,14,15], based on a very high prevalence of up to 100%. But, it is known that the pre-test probability in symptomatic patients, in clinical practice may be as low as 3-10% [10,16]. The performance of the AB assays at higher cut-offs for CD diagnosis are still lacking. Despite numerous efforts by the CD societies, there is no sufficient standardization and adequate reference protocols and no reliable quality assessment of the ELISA AB kits for CD diagnosis and follow-up of dietary adherence [17,18,19].

The following is the summary of limitations that are important to clinicians, laboratory staff, dieticians, researchers and others that are dealing with gluten sensitive conditions, concerning IgA-TG2 ABs [18]:

1. The diversity of available test kits, impeding comparisons between manufacturers and population studies.

2. The discrepancy between selective scientific validation, at the bench or bed environment and less "sterile", field originating or diverse populations.

3. The ABs reflect the activity of reactive but not innate immunity, or the intestinal inflammatory state of the CD intestinal mucosa.

4. The performance of the ABs is age dependent. In children they are more accurate for CD diagnosis. IgA-TG2 AB are more often negative in CD patients older than 70y [20].

5. They are not reliable in monitoring disease activity in GFD CD treated patients [18,19].

6. No data exist on the impact of the serological activities on long-term prognosis, complications, extraintestinal manifestations or autoimmunity genesis or progression.

7. In view of the multi-functions of the endogenous enzyme and its specific AB, the field of loss/enhancement of TG2 functions induced by IgA-TG2 autoantibodies is far from being explored. It is clear that the balance between the two and the AB levels will impact both disease establishment and progression.

8. The debate whether to use only IgA-TG2 or to combine a complementary diagnostic autoantibody, to improve diagnosis and follow-up performance, is as yet unresolved [2,5,12,21].

9. A discrepancy between manufacturer's cut-off and receiver operating characteristic plot derived cut-off values, modifies decision thresholds of the kits assays. More so, Kappa analysis demonstrates variable degrees of agreement [22]. Sometime this variability can reach 75% [20].

10. In young children and even in older ones, seropositivity of IgA-TG2, may be a transient phenomenon, and not necessarily predictive of CD development.

11. Several authors have raised questions regarding the diagnostic performance of IgA-TG2 ABs in routine clinical practice [23,24]. Several strategies have been suggested to improve performance or interpretation of the test, but these have not gained wide laboratory application [16,25,26].

In summary, IgA-TG2 AB is a highly reliable, non-invasive celiac diagnostic test. However, its reflection of the intestinal damage and monitoring of disease activity, is disappointing. It has multiple limitations and the debate as to whether it should be combined with an additional serological marker or replaced with a better marker is still ongoing. It seems that the currently available serological biomarkers' performances, like EMA or DGP, are not sufficient to replace IgA-TG2 AB [27,28], but recent data indicates that the neo-epitope TG2 AB is a prime candidate in this race [8]. Still, a combination test may prove better [2,5,10,11,12].

3. False Positive Anti IgA-TG2 ABs

Seropositivity has two main aspects: Positive IgA-TG2 in face of a normal small bowel biopsy in a definitive CD patient, or false positive ABs, in face of a non-CD condition.

3.1. Positive IgA-TG2 in Histological Normal Small Bowel Biopsy

Small bowel pathology can be Marsh1 grade, generally none specific, or normal, in face of positive serology [19,29]. The interpretation might be the patchy distribution of the disease, missed duodenal bulb pathology, more distal enteropathy, or latent CD. Sometimes, subepithelial IgA-TG2 complexes can be detected, if looked for, further substantiating the diagnosis.

3.2. True False Positive IgA-anti Human TG2 ABs

Several situations can be encountered in positive serology in none CD conditions:

1. Autoimmune diseases. False + anti TG2 AB have been widely described in celiac associated and none associated autoimmune disease [30,31,32]. Connective tissue disease, IBD, Primary biliary cirrhosis, Goodpasture's syndrome, Wegener's granulomatosis, rheumatoid arthritis, SLE, progressive systemic sclerosis, type 1 diabetes, IgA pemphigus, are some of them.

2. Non-autoimmune diseases. Interestingly, 6.1% of patients having diverse diagnoses but increased serum

IgM rheumatoid factor levels were found to be false-positive for anti TG2 ABs attesting to the fact that false positivity exists in none autoimmune serological conditions [33]. Additional examples are none autoimmune cirrhosis, linear IgA dermatosis, herpes gestationis, vasculitis, etc. Exploring the reasons for such false positivity, Sardy et al found that minor impurities in the recombinant human TG2 used and raised IgA antibody levels can explain the phenomenon [30].

3. When anti TG2 is positive, in the face of a negative EMA, the rate of CD is very low [34].

4. Transient positivity of anti TG2 ABs. Several groups reported on the natural longitudinal variation of serum levels of TG2 ABs in diabetic or genetic high risk CD young children or adolescents [35,36,37].

5. Children with cerebral palsy tend to have false positive anti TG2 and anti gliadin ABs. False positivity was associated with lower weight, height and BMI in those children [38].

6. In clinical practice, even strongly positive anti IgA-TG results (even >X10 UNL) are not specific in individual patients, do not necessarily correlate with the degree of severity of biopsy change and, as a result, are also unlikely to be useful for monitoring diet compliance [39].

7. Even during an infectious febrile illness, anti TG2 Abs are transiently detected in serum. The authors suggest that those ABs may not be produced by the intestinal mucosa [40].

4. False Negative Anti IgA-TG2 ABs in CD

IgA-TG2 seronegativity, per definition, will occur when the AB titer is negative in face of a pathological small bowel biopsy in a definitive CD patient. False negativity of serological IgA-TG2 ABs have been described in the following conditions:

1. Complete IgA deficiency. Much more prevalent in CD. Appears in 0.14-0.2% of the general population and in 2.6% of CD patients. However, low but detectable total IgA do not appear to affect the test specificity [19]. When the possibility of IgA deficiency exists, IgG based tests can circumvent the problem, but they are not suitable for monitoring dietary compliance.

2. Normal to low positive titers have been described in refractory CD and small-intestinal bacterial overgrowth [41].

3. Age dependency: 50% of CD patients >70 years old were negative for IgA-TG2 compared to 15% of the younger CD patients [42].

4. Intestinal autoantibody deposits in face of negative systemic antiTG2 ABs. TG2-targeted autoantibodies were deposited in small-bowel mucosa even when EMA and IgA-TG2 ABs were absent in the serum of CD patients [43]. These deposits proved to be the best predictor of subsequent CD development [44].

5. Gluten free diet. Many gluten sensitive populations start GFD deliberately. Such custom may jeopardize the performance of the gluten- dependent CD-associated ABs. GFD may induce false negative IgA-TG2 test.

6. The prevalence of CD is lower in primary care (0.5-1.0%) than in endoscopy units (1.0-5.2%), affecting the performance of the diagnostic flow chart. The prevalence

of CD with negative IgA-TG2 ABs was 0.4%, inan endoscopy set-up [45].

7. Seronegative CD is defined by the negativity of anti-TG2 ABs in the presence of pathological CD duodenal biopsies. A recent comparison of several studies reported the prevalence of anti-TG2 ABs in non-atrophic CD to be within the range of 0-88% [3]. Several potential explanations for this were suggested: 1. The mucosal deposits of the enzyme and its autoantibodies counteract the passage of the ABs into the bloodstream, 2. Incomplete maturation of the plasma cells or immunoglobulin deficiencies with a consequent failure of AB production [3].

8. Transient celiac serology or just temporary fluctuation? The levels can be high or low, depending upon the CD diagnosis/gluten intake, thus presenting as false positive/false negative, respectively [37,46]. Collin et al, reported on 4 children and adults with negative CD serology and normal intestinal biopsies, developing classical serological and pathological CD 2-17 years later [46].

9.In the face of negative anti Tg2 results, the titers are independently related to age and the indication for the serological testing, be it because of suggestive CD symptoms or high risk history [47].

In summary, the present review highlight and increases the awareness of the professional communities that screen, diagnose and follow CD, for the multiple circumstances of false +/- IgA-TG2 serology. It is hoped that it will help in making clinical decisions, since each year of delay in CD detection is associated with a significant increase in medical care costs. Its correct diagnosis can lead to considerable decrease in morbidity, mortality, and saving of both economic and medical resources [48].

5. Conclusions

The medical CD professionals are using routinely IgA-TG2 serology and face often the dilemma of its interpretation. Understanding the circumstances and the significance of the false positive/false negative anti-TG2 ABs will improve the clinical judgment of the CD associated health workers in face of a suspected CD patient. The information contained in the present reviews, hopefully, will stimulate the scientific community to explore these issues, and stimulate the diagnostic serology manufacturers to improve their product performance.

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