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Inter- and Intra-assay variation in the diagnostic performance of anti-tissue transglutaminase antibody assays in two populations

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Introduction

Tissue-transglutaminase-2 (TG2) based immunoassays are the cornerstone of diagnosis in celiac disease (CeD) with a reported pooled sensitivity as high as 98%¹. However, a few small, single-center studies have questioned their sensitivity in clinical practice^{2–5}.

Moreover, commercial kits use variable TG2 antigens⁶ with cut-offs determined using small, poorly-defined populations. Variation in diagnostic performance of anti-TG2 assays in different racial and geographic populations has not yet been studied. We compared the inter-assay and intra-assay variation in diagnostic performance of four IgA-anti-TG2 assays in Canadian and Indian populations.

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Methods

Patients with CeD were recruited from Manitoba Celiac Disease Cohort in Canada and Outpatient Gastroenterology clinic of a tertiary care center in India. CeD was diagnosed based on positive IgA-anti-TG2, modified Marsh grade 2 histology and an unequivocal response to a gluten-free diet. Controls were patients with functional gastrointestinal diseases who had negative IgA-anti-TG2 and Marsh 0 histology on a gluten-containing diet. For both cohorts, IgA-anti-TG2 was measured at each site using four commercially available assays (Table 1). The results of Celikey assay in India could not be interpreted due to software malfunction and were excluded from analysis. Using 90.2% sensitivity and 95.4% specificity as standard¹ (with 5% margin of error), we needed 138 patients with CeD and 73 controls. Sensitivity and specificity for each assay were calculated for both cohorts using manufacturer provided cut-offs. Area under ROC (AUROC) were compared and ROC-based cut-offs providing the best combination of sensitivity and specificity were calculated^{7,8}.

Results

We included 150 Indian and 140 Canadian patients with CeD along with 86 controls at each site. In Indian cohort, sensitivity was lowest for Euroimmun (76%) and highest (93.3%) for Aeskulisa (Table 1). In contrast, Euroimmun had the highest sensitivity among Canadian patients, and Aeskulisa had the lowest sensitivity (76.4%). AUC ranged from 0.88–0.91 in Indian cohort ($P=0.20$) and 0.88–0.97 in North American cohort ($P<0.001$). Among patients with CeD who had IgA-anti-TG2 levels 10 times upper limit of normal (ULN) ($n=169$) and 5ULN ($n=217$) on one of the assays, 0–4.1% and 1–7.4%, respectively, were falsely seronegative on another assay.

For Quanta Lite, Celikey and Euroimmun assays, seropositivity increased sequentially from Marsh 2/3a histology to Marsh 3c, e.g., Quanta Lite was seropositive for 57.9% with Marsh 2 and 87.1% with Marsh 3c histology. Excluding patients with Marsh 2 histology did not improve the sensitivity significantly. In multivariable analysis, study site and histologic severity affected IgA-anti-TG2 assay results while age and gender did not.

Adjusting the cut-off threshold using ROC plot analyses improved sensitivity for several assays without significantly compromising specificity; however, optimal cut-offs differed between the two populations (Table 1).

Discussion

In this head-to-head comparison of IgA-anti-TG2 assays in two racially distinct geographic populations, we found significant inter-assay and intra-assay variation in diagnostic accuracy. Sensitivity ranged from 76.4–97.9% in the Canadian cohort and 76–93.3% in the Indian cohort. Notably, the false negative rate was as high as 24% suggesting that a significant proportion of patients with CeD may be missed if a single negative IgA-anti-TG2 result is relied upon to rule out CeD in all cases. Moreover, our findings raise concern whether “seronegative CeD” is truly seronegative, or if it is seronegative on the assay used.

Although IgA-anti-TG2 10-fold ULN was 100% specific for CeD in our study, we observed that the high inter-assay variability is a concern even for this subgroup of patients. Among those with IgA-anti-TG2 10-fold ULN on one assay, up to 4% were seronegative on another assay. In clinical practice, these patients may be diagnosed with CeD based upon high IgA-anti-TG2, or, depending on the assay used at the laboratory where they happen to be tested, not be diagnosed with CeD if normal IgA-anti-TG2 testing is not followed by biopsy.

The assay with the highest sensitivity in the Indian population had the lowest sensitivity in the Canadian population and *vice versa*. Potential reasons include differential exposure to gliadin epitopes, differential antibody response and other unmeasured confounding factors between the two populations. Laboratory processing differences between two sites is possible but manufacturer's protocols were strictly followed. Thus, clinicians should exert caution while comparing the results of celiac serology based studies from different geographic populations.

In conclusion, the diagnostic performance of IgA-anti-TG2 assays varies significantly within and between two racially distinct geographic populations. In patients with high pretest probability of CeD, a single IgA-anti-TG2 assay may not adequately rule out CeD and duodenal biopsy should be strongly considered. Diagnostic performance of IgA-anti-TG2 assays cannot be assumed without assessment of their performance in specific populations.

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Abbreviations

CeD	Celiac disease
TG2	Tissue transglutaminase-2
ULN	Upper limit of normal
ROC	Receiver operator curve
AUROC	Area under receiver operator curve

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Sensitivity and specificity of four commonly used IgA-anti-TG2 assays in Indian and North American populations

Table 1:

IgA-anti-TG2 assay	Manufacturer cut-off	Indian population (150 CeD; 86 controls)	North American population (140 CeD; 86 controls)	Results using ROC obtained optimal cut-off values					
				Optimal cut-off	Sensitivity	Specificity	Optimal cut-off	Sensitivity	Specificity
Quanta Lite h-tTG IgA ¹	4 kU	76.7	87.9	2.25 kU	85	95	2 kU	100	98.8
Anti-Tissue Transglutaminase ELISA (IgA) ²	20 kU	76	97.9	9.8 kU	85	94	28 kU	96	99
AESKULISA tTG-A New Generation ³	18 kU	93.3	76.4	25.8 kU	89	98	3.8 kU	95	98
EliA Celikey tTG IgA ⁴	6.9 kU	N/A	90.7	N/A	N/A	N/A	2.7 kU	100	99

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