

Tissue transglutaminase antibody levels predict IgA deficiency

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ABSTRACT

Objective Measuring serum tissue transglutaminase immunoglobulin A (tTG IgA) levels is the most widely used screening test for coeliac disease. However, given an increased prevalence of IgA deficiency among coeliac patients there is a risk of false negative results. Hence, in addition to specific serum tTG IgA, screening tests frequently include total IgA levels. The objective of this study was to determine whether tTG IgA antibody levels might be used to predict IgA deficiency and hence avoid unnecessary testing of total IgA levels in all individuals.

Design Retrospective analysis of 9429 serum tTG IgA and corresponding total IgA levels obtained from children and young adults in the East of England between 2007 and 2011.

Results The overall prevalence of IgA deficiency was found to be very low with only 0.9% of individuals affected. Using receiver operating characteristic curve analysis we identified a cut-off value for tTG IgA of $\geq 0.10 \mu\text{g/mL}$ to be predictive for the absence of total IgA deficiency ($\text{IgA} < 0.06 \text{ g/L}$). Specifically, using this cut-off value, total IgA deficiency could be excluded with a sensitivity of 0.92 and specificity of 0.84. In our cohort, only 16.4% of our patient sample would have needed total IgA measurement to rule out a false negative result due to IgA deficiency.

Conclusions Our data provide a simple means of avoiding unnecessary total IgA measurements in the assessment of coeliac disease. By using tTG IgA value quantitatively, only values $< 0.10 \mu\text{g/mL}$ require total IgA measurements to rule out IgA deficiency and hence a potentially false negative screening result.

INTRODUCTION

Coeliac disease can be defined as an autoimmune disorder of the small intestine that occurs in genetically predisposed individuals. Symptoms include chronic diarrhoea, failure to thrive (in children) and abdominal pain. However, particularly outside infancy symptoms can be mild or even completely absent. The combination of easily available, reliable and minimally-invasive blood testing has led to increased screening of at risk individuals. Standard testing is currently based on measuring specific serum immunoglobulin A (IgA) antibodies either against endomysium and/or against tissue transglutaminase (tTG IgA), an enzyme known to play a crucial part in coeliac disease pathogenesis.¹ Serum tTG IgA has been shown to be highly sensitive in diagnosing coeliac disease in individuals with normal total IgA levels. However, selective IgA deficiency is not only the most common primary immunodeficiency in Caucasians (about 1 in 600),² but is even more prevalent in patients with coeliac disease at about 5%.³ IgA deficiency represents the

What is already known on this topic

- ▶ Measuring serum tTG (tissue transglutaminase) IgA levels is the most widely used screening test for coeliac disease.
- ▶ Given an increased prevalence of IgA deficiency amongst coeliac patients there is a risk of false negative results. Screening for coeliac disease therefore often routinely combines measuring specific tTG IgA as well as total IgA. The main reason for this is to avoid false negative results in IgA deficient patients.

What this study adds

- ▶ Our study demonstrates that tTG IgA can be used as a screening tool for coeliac disease in isolation.
- ▶ We provide a cut-off level for tTG IgA, which identifies individuals at risk of IgA deficiency that require subsequent total IgA measurement.
- ▶ By using tTG value quantitatively, only values $< 0.10 \mu\text{g/mL}$ require total IgA measurements to rule out IgA deficiency and hence a potentially false negative screening result.

most common cause for a false negative IgA tTG level in patients with coeliac disease.⁴ According to current UK NICE guidelines, all individuals tested negative for tTG IgA with a low or very low optical density should have a total IgA level measured to identify a potentially false negative coeliac test and document a primary IgA immunodeficiency.⁵ The clinical consequences of the latter are most often minimal, although recurrent upper and lower respiratory tract infections may occur.⁶ Hence the importance of documenting an absolute IgA deficiency in an otherwise asymptomatic individual is debateable. None the less, while some centres approach this problem by measuring serum IgG antibodies against deaminated Gliadin, in many centres routine testing for coeliac disease consists of simultaneous measurement of serum tTG IgAs and total serum IgA levels. However, given that only a minority of negative tTG IgA levels are due to IgA immunodeficiency, a large number of total IgA measurements are performed unnecessarily.

In this study, we analysed a large dataset of 9429 paired serum tTG and total IgA measurements with the aim of evaluating the predictive value of tTG IgA in identifying individuals with IgA deficiency.

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Results of our analysis provide a cut-off value for all tTG measurements below which a total IgA measurement is required to exclude true IgA deficiency and hence a false negative result. Importantly, applying this cut-off to our own cohort would make 80% of all our serum IgA measurements unnecessary, highlighting potentially significant cost savings.

METHODS

The Department of Biochemistry at Addenbrookes Hospital analyses coeliac serology for five district hospitals and primary care practices whose catchment population is 349 731 under 20 years of age (erpho.org.uk; catchment population by strategic health authority in 5-year age bands). The results of every coeliac serology analysis and concomitant total IgA are logged on a secure electronic database. The results of all samples analysed over 4 years (October 2007–November 2011) were exported into a separate database for analysis. Only patients after 6 months of age and before their 18th birthday were included in the analysis. We included only the first samples that were taken for each patient, with each sample therefore representing a single time point for a single patient. We could not differentiate whether a sample was taken for diagnostic screening or at a follow-up visit. However, given the 4-year duration of the study and the exclusion of subsequent samples, we assume the vast majority were samples sent for diagnosis of coeliac disease. Specific tTG IgA was tested using ImmunoCAP 250 (EliA Celikey IgA, Thermo Scientific) and total IgA was measured using laser nephelometry. All of these samples were tested in the same laboratory using the same, well-established assays.

During the study period, we received a total of 11 532 samples for coeliac screening consisting of combined testing for tTG IgA and total IgA antibodies. Of these, we excluded 2103 samples, which were repeat samples on the same patient(s). Choosing only the first available sample for each patient resulted in a total of 9429 individual patient samples being included in our analysis.

Data were then stratified into different subgroups for analysis. Given the age-specific differences in total IgA reference ranges (reference ranges were provided by the department of biochemistry and are currently being used in all major hospitals throughout the East of England),⁷ samples were divided into seven age groups based on the lower limit of the reference range of total IgA (table 1). Each age group was then further subdivided into three categories based on their total IgA levels (absent IgA<0.06 g/L across all age groups; low IgA>0.06 but < age-specific lower limit of reference range; normal IgA—within age-specific reference range). The threshold for IgA levels (ie, IgA<0.06 g/L) was determined by the minimum detection level for the IgA assay used in this study.

Table 1 Age dependant normal IgA reference values

Age	IgA (g/L)
6–9 months	0.15–0.7
9–12 months	0.2–0.7
1–2 years	0.3–1.2
2–3 years	0.3–1.3
3–6 years	0.4–2.0
6–9 years	0.5–2.4
9–12 years	0.7–2.5
12–15 years	0.8–2.8
>15 years	0.8–3.7

IgA, immunoglobulin A.

Statistical analysis

To investigate if low or absent IgA levels in individuals are related to low or absent tTG IgA levels, we tested for a correlation between total IgA and specific tTG IgA levels by calculating Kendall's τ -b correlation coefficients, stratified by age and IgA subgroup.

A receiver operating characteristic (ROC) curve of sensitivity against 1-specificity was then constructed in order to determine the optimum cut-off point of tTG IgA which can be used in the prediction of IgA deficiency (based on total IgA). This analysis was done both within each age group as well as including all samples irrespective of age. IgA deficiency was concluded if total IgA was less than 0.06 g/L across all age groups. The area under the ROC curve was also calculated in order to assess the overall diagnostic ability of tTG IgA in predicting IgA deficiency.

RESULTS

First we investigated the occurrence of absent and low IgA levels in the different age groups. Individuals were therefore divided into seven age groups and IgA levels grouped according to the age appropriate reference into 'absent IgA', 'low IgA' or 'normal IgA' levels (table 2). The overall prevalence of absent IgA was found to be very low with only 0.9% of all individuals affected, while 9.3% displayed low IgA levels.

Assuming that individuals with low or absent IgA levels would be more likely to have low or even absent tTG IgA levels, we next tested for a correlation between total IgA and specific tTG IgA levels by performing Kendall's τ correlation coefficients, stratified by age and IgA subgroup. As summarised in table 3, we observed a moderate, yet statistically significant correlation between total IgA and tTG IgA levels for the combined cohort of all age groups as well as for most individual age groups. Below the age of 6, however, there was no correlation between tTG IgA and total IgA in the low IgA group. Due to the minimal variability in absolute values in the patient group with absent total IgA levels, it was not possible to perform correlation analysis. Taken together, as expected, these data indicate that there is a moderate but yet statistically significant correlation between total IgA and specific tTG IgA levels in our patient population. Specifically, individuals with very low tTG IgA levels are more likely to have absent IgA levels, that is, be IgA deficient.

Next we aimed to identify a suitable cut-off value for specific tTG IgA levels, which could reliably exclude, or identify, individuals at high risk of being IgA deficient and hence who require total IgA levels. We therefore performed a ROC analysis both on individual age groups as well as the combined sample set (table 4). Values of specificity and sensitivity of tTG IgA levels

Table 2 Distribution of IgA levels among age groups

Age	Total	Absent IgA (% of total)	Low IgA (% of total)	Normal IgA (% of total)
6–8 months	111	4 (3.6%)	19 (17.1%)	88 (79.3%)
9–11 months	172	0 (0.0%)	41 (23.8%)	131 (76.2%)
1–2 years	1001	8 (0.8%)	155 (15.5%)	838 (83.7%)
3–5 years	1322	12 (0.9%)	82 (6.2%)	1228 (92.9%)
6–8 years	1361	14 (1.0%)	76 (5.6%)	1271 (93.4%)
9–11 years	1600	12 (0.8%)	168 (10.5%)	1420 (88.8%)
12–17 years	3862	36 (0.9%)	332 (8.6%)	3494 (90.5%)
Total	9429	86 (0.9%)	873 (9.3%)	8470 (89.8%)

IgA, immunoglobulin A.

Table 3 Correlation between total IgA and tTG IgA (μmL) levels

	Low IgA	Normal IgA	All patients
All age groups	0.35 (N=873, $p<0.001$)	0.50 (N=8470, $p<0.001$)	0.54 (N=9429, $p<0.001$)
Age groups			
6–8 months	–0.11 (N=19, $p=0.59$)	0.48 (N=88, $p<0.001$)	0.47 (N=111, $p<0.001$)
9–11 months	0.08 (N=41, $p=0.56$)	0.35 (N=131, $p<0.001$)	0.37 (N=172, $p<0.001$)
1–2 years	0.25 (N=155, $p<0.001$)	0.42 (N=838, $p<0.001$)	0.46 (N=1001, $p<0.001$)
3–5 years	–0.04 (N=82, $p=0.70$)	0.43 (N=1228, $p<0.001$)	0.46 (N=1322, $p<0.001$)
6–8 years	0.007 (N=76, $p=0.94$)	0.46 (N=1271, $p<0.001$)	0.49 (N=1361, $p<0.001$)
9–11 years	0.29 (N=168, $p<0.001$)	0.39 (N=1420, $p<0.001$)	0.47 (N=1600, $p<0.001$)
12–17 years	0.24 (N=332, $p<0.001$)	0.45 (N=3494, $p<0.001$)	0.50 (N=3862, $p<0.001$)

IgA, immunoglobulin A; tTG, tissue transglutaminase.

were also calculated and an optimal cut-off point was identified. As illustrated in table 4, results varied among groups with generally higher values of sensitivity, specificity and area under the curve found in older children. However, the optimum cut-off value for tTG IgA was found to be $<0.1 \mu\text{mL}$ in all the age groups providing an overall sensitivity of 92% and specificity of 84% for the combined samples (table 4 and figure 1). Together, these results indicate a high predictive value of serum IgA tTG levels to identify individuals with absent total IgAs.

Applying this cut-off value to our patient population (ie, the combined sample set), we identified 92% (79 out of 86) individuals with IgA deficiency (table 5). Moreover, in 99.9% of cases (ie, 7877 out of 7884) this cut-off reliably excludes IgA deficiency.

Having established a reliable cut-off value we next evaluated in what proportion of patients future testing could be avoided. Table 6 summarises the number and percentage of patients in each age group found to have tTG IgA values of $<0.1 \mu\text{mL}$. While the relative occurrence of low tTG IgA levels are found to be more frequent in younger age groups, overall only 16.4% of children and young adults had levels below our cut-off value of $0.1 \mu\text{mL}$. In other words, applying the cut-off value to the entire sample set would have saved a total of 9987 (7884 ($>0.1 \mu\text{mL}$) and 2103 (repeat samples)) IgA tests. Given the current price of performing a total IgA measurement of £2.90, adhering to our suggested guidance could lead to savings of approximately £7200 per annum.

DISCUSSION

Testing for coeliac disease has increased over the past decade, particularly in the paediatric population. This is well demonstrated by our own data that show a 100% increase in tTG IgA

requests, in a relatively stable population, over the period of our study. This is mainly due to our increased understanding of a changing disease spectrum, with the majority of affected individuals now presenting with a wide variety of mild to moderate, often non-specific, clinical symptoms. Children and young adults who are tested generally fall into two groups: the first are symptomatic individuals who are suspected of having coeliac disease, and the second are asymptomatic individuals with an inherent increase in risk of having the disease. The former includes children with chronic abdominal pain, chronic constipation, diarrhoea, poor weight gain and growth failure, while asymptomatic screening occurs more frequently in individuals with pre-existing auto-immune disease, a positive family history, trisomy 21 and others. Together, this represents a potentially large proportion of the paediatric patient population; in our study, about 3% of the potential population (9429/349 731) had a blood test during the study period. Despite the fact that testing for serum IgA tTG has been shown to be highly sensitive¹ the risk of a false negative result due to selective IgA deficiency currently requires additional testing with a total IgA level. A large multi-centre prospective study (ProCeDe—see below) is currently underway to validate the recent ESPGHAN guidelines on coeliac disease,⁸ which have raised the possibility that not all children may require diagnostic duodenal biopsies to confirm a diagnosis of coeliac disease. This study should provide definitive evidence what panel of serological and genetic assays are sensitive and specific enough to avoid duodenal biopsy.⁹

In our study, we set out to investigate whether only the IgA tTG test result is able to reliably predict the presence of IgA deficiency. Our data illustrate that with a threshold of $>0.1 \mu\text{mL}$ of IgA tTG, total IgA deficiency can be reliably excluded in 99.9% of cases. Importantly, only 16.4% of all tested

Table 4 Summary of ROC analysis in individual age groups

	Optimum cut-off point for tTG (μmL)	Sensitivity	Specificity	Area under ROC curve (95% CI)
All age groups	<0.10	0.92	0.84	0.876 (0.841 to 0.911)
Age groups				
6–8 months	<0.10	1.00	0.34	0.668 (0.469 to 0.868)
9–11 months*	NA	NA	NA	NA
1–2 years	<0.10	1.00	0.56	0.780 (0.690 to 0.871)
3–5 years	<0.10	0.92	0.76	0.832 (0.729 to 0.934)
6–8 years	<0.10	0.86	0.87	0.835 (0.704 to 0.966)
9–11 years	<0.10	0.83	0.90	0.857 (0.734 to 0.980)
12–17 years	<0.10	0.94	0.94	0.949 (0.917 to 0.980)

*No patients aged 9–11 months with absent IgA identified.

IgA, immunoglobulin A; ROC, receiver operating characteristic; tTG, tissue transglutaminase.

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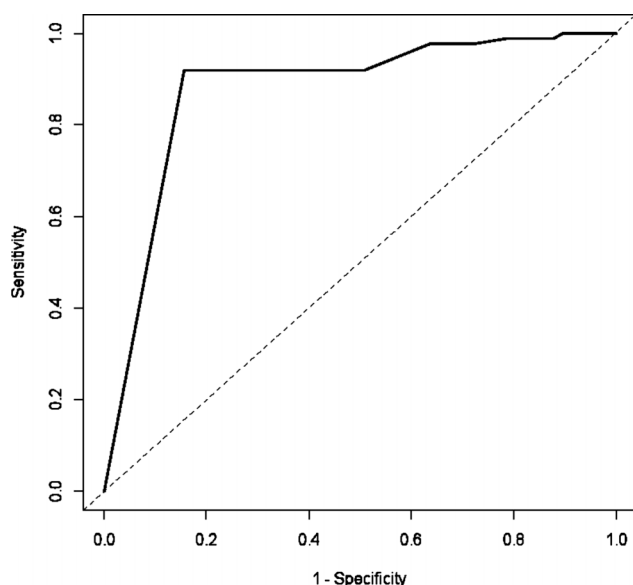


Figure 1 Receiver operating characteristic (ROC) analysis to assess predictive value of tissue transglutaminase (tTG) immunoglobulin A (IgA) in identifying IgA deficiency. A ROC curve of sensitivity against 1-specificity was constructed in order to determine the optimum cut-off point of tTG IgA which can be used in the prediction of IgA deficiency (based on total IgA <0.06 g/L). IgA deficiency was concluded if total IgA was less than 0.06 across all age groups. The area under the ROC curve was calculated in order to assess the overall diagnostic ability of tTG IgA in predicting IgA deficiency.

individuals were found to have a tTG IgA level of <0.1 µ/mL, which would then require the measuring of a total IgA.¹⁰ Given the current financial climate of healthcare systems, reducing expense by avoiding unnecessary investigations is an important part of improving efficiency without compromising quality of care. Applying this cut-off value to the total patient population would have reduced the number of total IgA measurements by 9987, which would have resulted in a total saving of £28 962 over 4 years. It is of course important to emphasise that the type of laboratory tests, as well as reference and quality standards, may vary between institutions and hence local thresholds/cut-off values may apply.

A limitation of our retrospective study is that we could not validate these results against the clinical outcomes in this patient population. However, this study did not set out to assess the

Table 6 Number and percentage of patients with tTG IgA levels <0.1 µ/mL divided into age groups

Age	Total patients	No. of patients with tTG IgA <0.10 µ/mL	% of patients with tTG IgA <0.10 µ/mL
6–8 months	111	75	67.6%
9–11 months	172	101	58.7%
1–2 years	1001	444	44.4%
3–5 years	1322	320	24.2%
6–8 years	1361	191	14%
9–11 years	1600	163	10.2%
12–17 years	3862	251	6.5%
Total	9429	1545	16.4%

sensitivity of tTG IgA in the diagnosis or follow-up of children with coeliac disease, but rather to focus on whether a single quantitative test result (tTG IgA) could predict IgA deficiency with reasonable certainty.

CONCLUSIONS

In conclusion, based on over 9000 paired total IgA and tTG IgA, we demonstrate a reliable cut-off value for tTG IgA of 0.1 µ/mL, above which additional testing of total IgA, in children of any age group, may no longer be necessary. Only below this value is additional total IgA measurement still necessary to avoid a potentially false negative IgA tTG IgA result. Implementation of such recommendations in the screening for coeliac disease could lead to significant cost savings for the healthcare economy.

Contributors RBH, MZ, AS conceptualised and designed the study, drafted the initial manuscript, and approved the final manuscript as submitted. AS was also involved in data collection and analysis. RP carried out statistical analyses, reviewed and revised the manuscript, and approved the final manuscript as submitted. RM was involved in designing the study, data collection and analysis, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Competing interests None.

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REFERENCES

- Giersiepen K, Lelgemann M, Stuhldreier N, *et al.* Accuracy of diagnostic antibody tests for coeliac disease in children: summary of an evidence report. *J Pediatr Gastroenterol Nutr* 2012;54:229–41.
- Pan-Hammarstrom Q, Hammarstrom L. Antibody deficiency diseases. *Eur J Immunol* 2008;38:327–33.
- Wang N, Shen N, Vyse TJ, *et al.* Selective IgA deficiency in autoimmune diseases. *Mol Med* 2011;17:1383–96.
- Latiff AH, Kerr MA. The clinical significance of immunoglobulin A deficiency. *Ann Clin Biochem* 2007;44:131–9.
- CG86 Coeliac disease: NICE guideline. <http://guidance.nice.org.uk/CG86/NICEGuidance/pdf/English>
- Janzi M, Kull I, Sjöberg R, *et al.* Selective IgA deficiency in early life: association to infections and allergic diseases during childhood. *Clin Immunol* 2009;133:78–85.
- Milford Ward A. Protein reference unit handbook of clinical immunochemistry. In: Milford Ward A, Riches PG, Williams PE, (eds). 7th edition, p 131.
- Husby S, Koletzko S, Korponay-Szabo IR, *et al.* European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. *J Pediatr Gastroenterol Nutr* 2012;54:136–60.
- ProCeDe Study. <http://procede2011.jimdo.com/>
- Sinclair D, Saas M, Turk A, *et al.* Do we need to measure total serum IgA to exclude IgA deficiency in coeliac disease? *J Clin Pathol* 2006;59:736–9.

Table 5 Total number of individuals identified with absent IgA using a cut-off value of <0.10

		Absent IgA		Total
		No	Yes	
tTGA (µ/mL)	≥0.10	7877	7	7884
	<0.10	1466	79	1545
Total		9343	86	9429

IgA, immunoglobulin A.



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