Highs and lows of coeliac screening

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ABSTRACT

Coeliac screening is one of the most frequently requested investigations in immunology laboratories. This study evaluates seven commercial anti-tissue transglutaminase (tTG) enzyme-linked immunosorbent assay (ELISA) kits across a varied population of 80 routine samples received for coeliac screening. This investigation assesses whether or not absorbance levels can be used to detect low serum IgA and whether or not raised IgA concentrations can interfere with IgA-tTG results. Sensitivity and specificity varied hugely and four out of the seven methods required cut-off value modification to attain 100% sensitivity with endomysial testing. Four of the seven kits identified low IgA samples with greater than 90% accuracy, but sensitivity dropped to 75% in others. All the kits were affected by raised serum IgA concentrations, leading to false-positive results. There was notable variation between the seven kits in respect of high IgA concentrations and positive IgA-tTG results, with concordance analysis indicating a weak linear relationship between IgA concentration and tTG value. This study concludes that there is significant variability between the commercial tTG assays in the diagnostic market. Laboratories should be aware of their kit's limitations and may need to adjust cut-off values to maximise sensitivity. It is possible to identify IgA deficiency from the tTG values, but the ability to do this varies between manufacturers. Raised IgA levels continue to affect the specificity of IgA-tTG assays and interference by polyclonal and monoclonal IgA should be considered in samples with positive tTG and negative endomysial results.

KEY WORDS: Celiac disease.
Endomysial antibody.
Immunoglobulin A.
Transglutaminases.

Introduction

Coeliac disease (CD) is an autoimmune condition that occurs in genetically susceptible individuals and causes lifelong intolerance to dietary gluten.[1–3] Affecting as many as one in 87 people in the UK population,[4–6] it is controlled by strict adherence to a gluten-free diet that allows the small intestinal mucosa to recover.[7] The disease often goes undiagnosed in many countries where this small bowel enteropathy is most prevalent.[8,9]

Since the mid-1950s,[9] CD has been diagnosed by performing small intestinal biopsy to identify characteristic mucosal lesions.[10] This highly invasive procedure called for an improvement in diagnostic technique and thus an enzyme-linked immunosorbent assay (ELISA) to the wheat protein gliadin was developed in the 1970s.[11] In the late 1980s, the use of an indirect immunofluoresence technique to identify IgA-endomysial antibodies (EMA; usually performed on distal sections of primate oesophagus) was implemented and proved to have greater sensitivity and specificity for CD.[5]

In 1997, tissue transglutaminase (tTG) was identified as the primary target antigen recognised by EMAs in CD.[10] Since this discovery, IgA-tTG ELISA has been developed and has been shown to have high sensitivity and specificity for CD.[12] IgA-tTG has become the initial serological screen for CD in the majority of diagnostic laboratories, often 'confirmed' by EMA and followed by duodenal biopsy.

First-generation assays using guinea pig liver tTG lacked sensitivity and specificity[10] and were soon replaced by recombinant or purified human tTG (h-tTG), which performed with vastly improved diagnostic accuracy.[13] Various studies have compared second-generation tTG assays but draw contradictory conclusions. One study of 10 commercially available kits reports excellent diagnostic performance with minor differences in sensitivity and specificity.[10] Another reports high diagnostic accuracy between normal and abnormal test samples but refutes published cut-off values associated with the kits.[14]

Selective IgA deficiency is found in approximately one in 600 of the population,[15] although it is reported to be 10–20 times more common in patients with coeliac disease.[16] Hence, it is suggested[6,13,14] that total serum IgA should be measured to exclude a co-existing IgA deficiency that could lead to false-negative IgA-tTG results. Local observations, and those of others,[16,17] suggest that it may be possible to identify IgA-deficient patients by observing the results from the IgA-tTG assay. Establishing that patients with IgA deficiency have extremely low IgA-tTG results, due to the lack of background interference from serum IgA, should minimise the false-negative rate without the need to 'blanket' measure IgA concentration.

Equally, false-positive anti-tTG results are also seen in cases that are EMA- and biopsy-negative, with no other indication of CD. Often, these sera have raised IgA levels, which suggests that abnormal/polymeric IgA may be interfering, leading to false-positive results. One study[18] reported that the degree of false positivity due to hypergammaglobulinaemia is highly variable between

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commercial kits. There is also much debate surrounding interference of IgA-tTG assays from patients with liver diseases such as primary biliary cirrhosis (PBC). One study reports 26 false-positive IgA-tTG results out of 105 PBC patients tested.[19,20]

The majority of IgA in the circulation is IgA1, which consists of approximately 90% monomeric IgA and 10% polymeric IgA. The amount of polymeric IgA in the circulation has been shown to increase in liver disease.[18] Formulating a link between raised IgA and false-positive anti-tTG results could reduce the number of unnecessary small-intestinal biopsies.

At present, a number of patients are still subjected to duodenal biopsy to aid the diagnosis and confirmation of CD; however, following the introduction and continued improvement of anti-tTG assays, the need to biopsy patients may be diminishing. It is therefore necessary to assess the reliability of anti-tTG assays for diagnostic use.

Hence, despite the high diagnostic accuracy of IgA-tTG assays, various factors affect clinical interpretation. This study investigates the strengths and weaknesses of a sample of commercially available kits.

Materials and methods

Although small-intestinal histology is the current gold standard diagnostic test for CD it is widely recognised that relying on duodenal biopsies may be problematic.[21] Serological tests have a role in the diagnostic algorithm, with the combination of IgA-tTG and IgA-EMA conferring 98% diagnostic accuracy.[22] For the purpose of this evaluation, IgA-EMA was considered the laboratory gold standard.

Study population

Eighty serum samples received in the laboratory for routine CD screening between February and July 2007 were included in the study. Clinical details varied and included suboptimal weight, irritable bowel syndrome, fatigue and liver disease. The majority of patients had clinical suspicion of CD although 28 requests gave no clinical details.

The study population consisted of 46 females and 34 males (age range: 1–79 years). The 80 study samples were selected according to four agreed, predefined subgroups using the laboratory's current methodology (detailed below). The first subgroup consisted of 20 serologically negative sera (tTG-negative, EMA-negative, normal IgA [Group 1]). The second subset consisted of 20 sera with raised IgA concentration (tTG-positive, EMA-negative, IgA >4 g/L [Group 2]). The third subset consisted of 20 sera with low IgA (tTG-negative, EMA-negative, IgA <0.25 g/L [Group 3]). The fourth subset consisted of 20 serologically positive samples (tTG-positive, EMA-positive, normal IgA level [Group 4]) (Table 1). All 80 samples were categorised and anonymised. During sample collection the sera were aliquoted and stored at –35°C.

IgA-tTG ELISA

All 80 samples had previously been analysed on the Triturus analyser (Grifols, Cambridge, UK) using the D-tek IgA-tTG kit (Mons, Belgium). Six commercial companies supplied IgA-tTG kits for the study: Immco (Buffalo, NY, USA), Euroimmun (Lubeck, Germany), Aeskulisa (Wendelsheim, Germany), Celikey (Phadia, Uppsala, Sweden), Orgentec (Orgentec Diagnostika, Mainz, Germany) and The Binding Site (Birmingham, UK). The Triturus analyser was programmed according to the manufacturer's instructions for each of the six IgA-tTG ELISA kits. All 80 sera had tTG measured and quantified using the individual cut-off values stated by the suppliers.

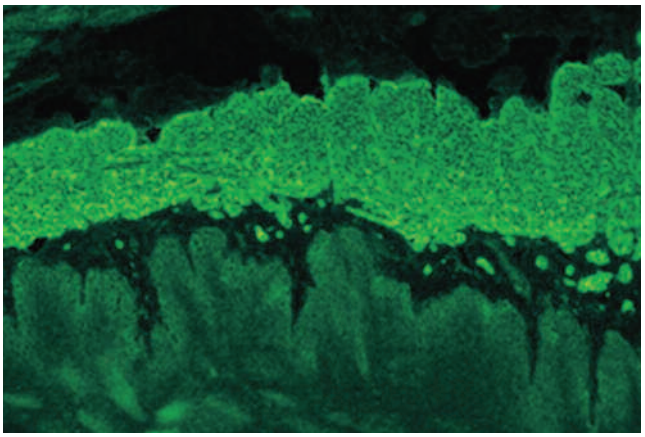

Fig. 1. Anti-EMA-positive (chicken wire pattern) staining of the muscularis mucosae on distal primate oesophageal tissue (*Atlas of tissue antibodies* 3rd edn. The Binding Site).

IgA concentration

The IgA concentration of each of the 80 samples was measured by rate nephelometry (BN Prospec, Dade Behring) with results reported in g/L. Following satisfactory calibration and quality control measurements, the IgA concentrations were established for all but four (low-volume) samples.

IgA-EMA and IgG-EMA

Anti-EMA antibody tests were performed by indirect immunofluorescence. Samples were diluted (1 in 5) in phosphate-buffered saline (PBS, pH 7.2). Slides with primate oesophagus sections were used as the substrate (Immco) and incubated with IgA anti-human polyclonal rabbit conjugate (Dako, Ely, UK), diluted (1 in 20) with PBS or IgG anti-human polyclonal rabbit conjugate (Dako) diluted (1 in 80) with PBS. The samples were evaluated for the presence of positive 'chicken wire' staining in the muscularis mucosae by two experienced biomedical scientists who were unaware of the tTG results.

Table 1. Four predefined subgroups chosen for the study.

	Anti-tTG	Anti-endomysial	IgA concentration (g/L)
Group 1	Negative	Negative	Within normal range for age
Group 2	Positive	Negative	Raised IgA (>4)
Group 3	Negative	Negative	Low IgA (<0.25)
Group 4	Positive	Positive	Within normal range for age

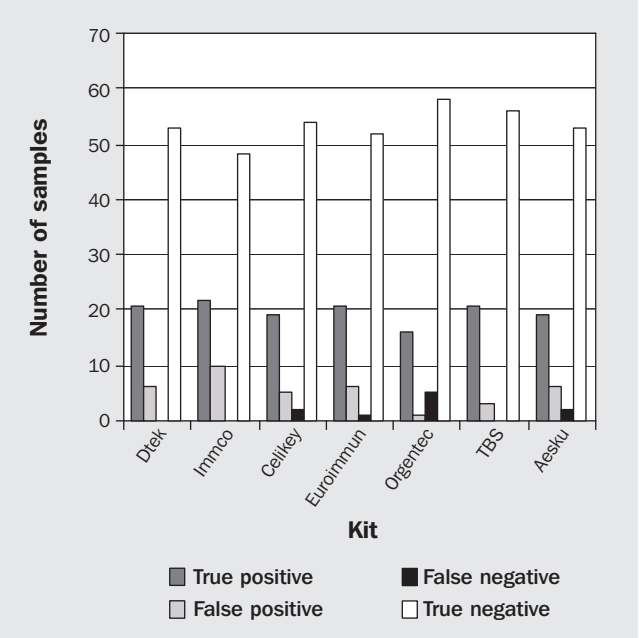

Fig. 2. Raw data from the 80 samples for seven kits. These data were used to calculate sensitivity, specificity and PPV.

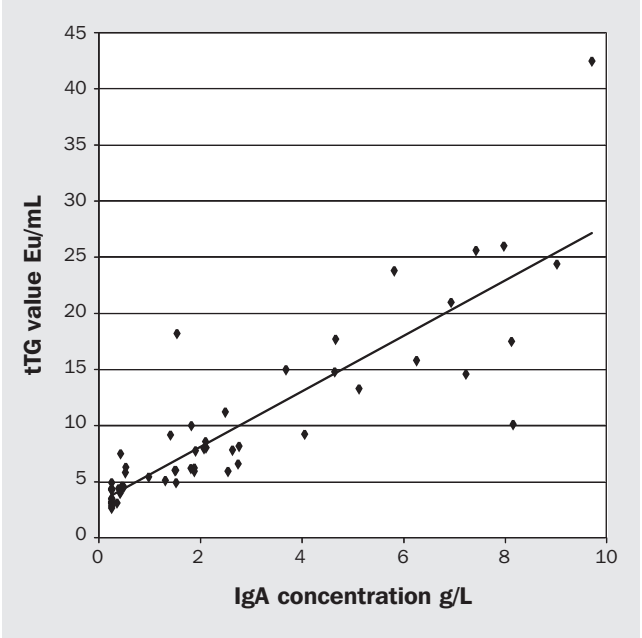

Fig. 3. IgA concentration against IgA anti-tTG result obtained from the Immco kit (r^2 = 0.7532, P=0.056).

IgG-tTG/IgG-gliadin ELISA

The 80 samples were analysed for IgG-tTG antibodies using the same instrumentation using The Binding Site IgG-tTG kit. Study samples with an IgA concentration <0.25 g/L were reflex-tested for the presence of IgG-gliadin antibodies by ELISA on the Triturus analyser using the D-Tek IgG-gliadin kit. This commercial kit was programmed to run according to the manufacturer's recommendations.

Data analysis

Data were stored in Excel spreadsheets (Microsoft Office XP Professional, version 10). Quantitative and qualitative results were recorded to aid comparison of data and identification of trends. Sensitivity and specificity results were calculated using the electronic patient record (EPR) toolkit (www.hutchon.net/EPRval.htm).

External quality assurance

The laboratory participates in the UK NEQAS schemes for total IgA concentration, IgA-tTG, IgA-EMA and IgG-gliadin assays.

Results

Diagnostic performance of tTG ELISA

Diagnostic sensitivities and specificities were calculated for each tTG assay using the EPR-validity test calculator. The IgA-EMA results were used to define serological positivity (Table 2) and this is represented in Figure 2.

All 20 serologically negative control samples (Group 1) were negative for IgA-tTG with all seven kits. Only three of the seven kits identified all 20 EMA-positive samples (Group 4) using the suggested cut-off. Of the four kits that failed to identify the EMA-positive sera, false negativity ranged from 1/20 (5%) to 5/20 (25%).

Based on the premise that IgA-tTG kits are used as a screening tool, four of the published cut-off values were lowered in order to achieve 100% sensitivity for all methods. The corresponding effects on assay specificity are shown in Table 2. Adjustment of the cut-off achieved 100% sensitivity to EMA for all kits; however, one kit (Euroimmun) showed no decrease in specificity. In two kits (Phadia and Orgentec), sensitivity was greatly improved with only a moderate

Table 2. Sensitivity and specificity for each of the seven kits using the manufacturer's cut-off value and amended cut-off value.

Kit	Units	Manufacturer advised cut-off	Sens. for EMA	Spec. for EMA	Amended cut-off	Sens. (amended cut-off)	Spec. (amended cut-off)
Immco	Eu/mL	25	100	83			
D-tek	u/mL	20	100	90			
Phadia	u/mL	5	90	92	3	100	90
Orgentec	u/mL	9	76	98	3.5	100	91
Aesku	u/mL	14	90	90	7	100	72
Euroimmun	Ru/mL	19	95	90	18	100	90
TBS	u/mL	4	100	95			

Table 3. Sensitivity and specificity of low IgA detection for each kit.

Kit	Units	Cut-off value for low IgA detection	Sensitivity for low IgA (%)	Specificity for low IgA (%)
Immco	Eu/mL	<4	100	98
D-tek	u/mL	<5	94	93
Phadia	u/mL	<0.5	85	93
Orgentec	u/mL	<1	95	85
Aesku	u/mL	<2	85	80
Euroimmun	Ru/mL	<1	75	80
TBS	u/mL	<0.25	95	95

decrease in specificity (2% and 6%, respectively), with Orgentec dramatically increasing its sensitivity from 76% to 100%. The remaining kit (Aesku) forfeited its specificity (90% decreased to 72%) in order to achieve 100% sensitivity (Table 2).

Detecting IgA deficiency

Nineteen out of the 20 sera with low IgA concentration gave a negative IgA-EMA and were negative for IgA-tTG by all seven kits. The remaining sample was interpreted as tTG-positive in one of the trial kits (Aesku), which was the only kit to have gliadin peptides incorporated in the antigen preparation. This patient also had an IgG-gliadin result of >400 iu/mL (normal range: 0–50 iu/mL).

The hypothesis that low IgA level could be identified using the absorbance (*A*) results of the assays was tested by studying the values from the 20 low-IgA samples and selecting the highest *A* within that group and translating this into a unit value. The selected level for each kit was then used to calculate the sensitivity and specificity of using that value to screen for IgA deficiency (Table 3). Figure 2 shows the unit values for each of the kits below which IgA deficiency was considered.

The IgA-tTG assays showed variability in their ability to detect IgA deficiency by using a selected unit value. Table 3 demonstrates the effectiveness of employing this method for each of the assays. The breadth of results indicates that low IgA detection is possible using some commercial kits but not others. Samples identified as having a low IgA concentration would benefit from further investigation using IgG assays.

Interference from raised IgA

Of the 20 samples with raised IgA, one (previously recorded as negative) gave a weak-positive IgA-EMA result and was therefore reclassified as having positive serology (Group 4). In the remaining 19 samples, false positivity ranged from 1/19 (5%) to 10/19 (53%). These data suggest that there may be a linear relationship between IgA concentration and positive IgA-tTG value. Figure 3 illustrates this relationship using IgA concentration and anti-tTG results taken from the Immco kit and shows only partial linearity (r^2=0.7532, P=0.056).[23] Other kits gave similar results (and r^2 values) to the example used here (data not shown).

Global agreement between the different methods was low, demonstrating that false positivity is related to different factors. Indeed, only one of the 20 sera was positive by all methods.

Table 4. Number of samples identified as IgA-tTG-positive by each kit employed and the false-positive rate as a percentage.

Kit	Positives (n=19)	False-positive rate (%)
D-tek	6	32
Immco	10	53
Phadia	5	26
Euroimmun	6	32
Orgentec	1	5
TBS	3	16
Aesku	5	26

IgG assays

The IgG-tTG assay was positive for 2/20 (10%) of the negative sera (Group 1), both of which were negative for IgG-gliadin and IgG-EMA assays. Twelve (60%) of the positive sera (Group 4) were positive for IgG-tTG. The low-IgA group and high-IgA group gave 2/20 and 4/20 positives, respectively, for IgG-tTG. It is notable that one of the samples from the low-IgA group had a strong positive IgG-gliadin (>400 iu/mL) and was also positive for IgA-tTG in the kit containing tTG and gliadin peptides, but was negative with the IgG-tTG and IgG-EMA assays.

Discussion

This study evaluated seven commercially available IgA-tTG ELISA kits. Unexpectedly, a huge variation in sensitivity and specificity was found (sensitivity 76–100%, specificity: 83–98%) using manufacturers' suggested cut-offs. Crucially, as IgA-tTG kits are used as a screening tool for CD, sensitivities below 100% were considered unacceptable. In four of the seven kits, the cut-off value was reduced in order to achieve 100% sensitivity with the IgA-EMA assay (Table 2). Contrary to previous reports,[9,10] concordance between methods was poor. These findings have highlighted the need for laboratories that perform IgA-tTG to evaluate their kit and consider whether or not the manufacturer's stated cut-off value may need to be altered.

In order to exclude co-existing IgA deficiency, it is suggested[5,24] that a total serum IgA concentration be measured. An objective of the present study was to identify sera with low IgA using the *A* results from the IgA-tTG ELISA. IgA measurements are expensive and incur significant additional cost to the laboratory, and reliable

identification of this subpopulation should be undertaken using IgG-gliadin, tTG or EMA assays to aid the diagnosis.

A cut-off value for each assay was determined and sensitivity/specificity data for detection of low IgA were calculated. Some kits were able to identify sera with low IgA concentrations, while others struggled (sensitivity for low serum IgA: 75–100%). These data are in agreement with previous studies[5,15] and illustrate that it is possible to utilise ELISA results to identify samples which require further investigation, but accuracy varies between kits.

It can be argued that not every sample in the reduced IgA subgroup was clinically IgA-deficient; however, a level <0.25 g/L (criterion for inclusion) was considered low enough to achieve the objective. One sample with clinical details of 'suboptimal weight' had an IgA concentration <0.25 g/L, negative anti-EMA and a negative tTG on all but one of the kits (Aesku; incorporating gliadin peptides). A very strong IgG-gliadin result was obtained on this sample (>400 iu/mL, normal range: 0–50 iu/mL). Clinical follow-up identified this patient as probable CD, but small-intestinal biopsy will be required to confirm the diagnosis.

Although studies indicate the potential of gliadin peptide-enhanced tTG assays,[25] this must be considered in view of the relatively poor specificity (Table 2) of this kit. A sample received from a 38-year-old female with a history of diarrhoea was selected for Group 4 (tTG/EMA-positive), and had a history of strong-positive IgA-EMA with a previous IgA-tTG >400 iu/mL (normal range: 0–50 iu/mL). All the commercial kits used in this study identified this sample as anti-tTG-positive, including the IgG-tTG kit. For completion of the database, an IgA concentration was performed on this sample and gave an IgA <0.25 g/L. Sensitive IgA assays confirmed the actual IgA concentration as 0.24 g/L. Although not truly IgA-deficient, it is possible that the majority of the 0.24 g/L IgA was coeliac-specific[26,27] and resulted in strongly positive coeliac serology despite a relatively low level of circulating IgA.

Conversely, raised IgA levels were also found to interfere with IgA-tTG assays and can yield false-positive results.[18,28,29] In comparison to EMA, false positivity in the raised IgA group (Group 2) ranged from 1/19 (5%) to 10/19 (53%). This subgroup of samples clearly poses a problem (to varying degrees) to the IgA-tTG assays assessed. Kit manufacturers have attempted assurance that the new-generation assays are superior to their predecessors in minimising interference from raised IgA levels and liver disease, but the findings of the present study show that raised IgA remains a problem.

Despite the diagnostic sensitivity of EMA, some studies have reported cases of antibody negative CD and tTG-positive, EMA-negative CD. In this study, one sample with raised IgA gave a positive tTG result with each of the seven kits and, despite being negative for IgG-tTG, gliadin and IgA-EMA, it must be considered that this patient is a possible CD. However, in contrast with a previous study,[10] we conclude that any positive IgA-tTG results will benefit from confirmatory IgA-EMA testing, as this will assist in the identification of false-positive IgA-tTG sera.

With regard to negative anti-EMA results, it has been suggested that negative serum EMA may be associated with advanced CD because transglutaminase 2 autoantibodies (TG2) are deposited in the small bowel mucosa.[30–33] Other opinions[10] dispute the need to perform IgA-EMAs; however, in the present study and another[18] the laboratory gold standard test has proved invaluable in confirming tTG serology and identifying possible false-positive reactions.

One limitation of the present study was the size of the sample population. Study size was restricted as it relied heavily on the generosity of commercial companies to provide kits. The broad objectives of this study compromised the sample size of each group. A further restriction to this study was that members of the subgroup selected for low IgA concentration were recruited when the laboratory technology was unable to measure IgA concentration accurately below 0.25 g/L. Viewed positively, being able to identify samples with low IgA concentration automatically ensures that true IgA deficiency will not be missed. The context of IgA concentration should also be considered, as an individual who is in a post-viral state may show transient IgA immunodeficiency.[19,34,35]

In conclusion, this study has highlighted the fact that low IgA concentration and raised polyclonal IgA concentration can influence the output from second-generation anti-tTG assays. Therefore, when considering a new assay, it is imperative that each laboratory identifies its quality targets and the kit's ability to achieve them. Hence, our experience suggests that a thorough and robust inspection of any new methodology is required to examine clinical utility.

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References

1 McGough N, Cummings JH. Coeliac disease: a diverse clinical syndrome caused by intolerance of wheat, barley and rye. *Proc Nutr Soc* 2005; **64** (4): 434–50.
2 Ludvigsson JF, Eylert M, Ilonen J *et al*. Effect of HLA DQ2, dietary exposure and coeliac disease on the development of antibody response to gliadin in children. *Scand J Gastroenterol* 2006; **41**: 919–28.
3 Karinen H, Karkkainen P, Pihlajamaki J *et al*. HLA genotyping is useful in the evaluation of the risk for coeliac disease in the 1st degree relatives of patients with coeliac disease. *Scand J Gastroenterol* 2006; **41**: 1299–304.
4 Diosdado B, WapenaarMC, Franke L *et al*. A microarray screen for novel candidate genes in coeliac disease pathogenesis. *Gut* 2004; **53**: 944–51.
5 Sinclair D, Saas M, Turk A *et al*. Do we need to measure total serum IgA to exclude IgA deficiency in celiac disease? *J Clin Pathol* 2006; **59**: 736–9.
6 Thomas PD, Forbes A, Green J *et al*. Guidelines for the investigation of chronic diarrhoea. *Gut* 2003: **52**: v1–15.
7 Scherer JR. Celiac disease. *Drugs Today (Barc)* 2008; **44** (1): 75–88.
8 Ludvigsson JF, Ludvigsson J. Coeliac disease in the father affects the newborn. *Gut* 2001; **49**: 169–75.
9 Hill PG, McMillan SA. Anti-tissue transglutaminase antibodies and their role in the investigation of celiac disease. *Ann Clin Biochem* 2006; **43**: 105–17.
10 van Meensel B, Hiele M, Hoffman I *et al*. Diagnostic accuracy of ten second-generation (human) tissue transglutaminase antibody assays in celiac disease. *Clin Chem* 2004; **50**: 11.

11 Shoenfeld Y, Gershwin ME, Meroni PL. Anti-tissue transglutaminase and anti-endomysial antibodies. In: Shoenfeld Y, Gershwin ME, Meroni PL eds. *Autoantibodies* 2nd edn. London: Elsevier, 2007: 443–50.

12 Dieterich W, Ehnis T, Bauer M *et al*. Identification of tissue transglutaminase as the autoantigen of celiac disease. *Gastroenterology* 1998; **3**: 797–801.

13 Setty M, Hormaza L, Guandalini S. Celiac disease: risk assessment, diagnosis and monitoring. *Mol Diagn Ther* 2008; **12** (5): 289–98.

14 Hill ID, Dirks MH, Liptak GS *et al*. Guideline for the diagnosis and treatment of coeliac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr* 2005; **40**: 1–19.

15 Hill PG, Forsyth JM, Semeraro D, Holmes GKT. IgA antibodies to human tissue transglutaminase: audit of routine practice confirms high diagnostic accuracy. *Scand J Gastroenterol* 2004; **11**: 1078–82.

16 Abdul Latiff AH, Kerr MA. The clinical significance of immunoglobulin A deficiency. *Ann Clin Biochem* 2007; **44**: 131–9.

17 Johnston SD, McMillan SA, Collins JS, Tham TC, McDougall NI, Murphy P. A comparison of antibodies to tissue transglutaminase with conventional serological tests in the diagnosis of coeliac disease. *Eur J Gastroenterol Hepatol* 2003; **15** (9): 1001–4.

18 Villalta D, Crovatto M, Stella S *et al*. False-positive reactions for IgA and IgG anti-tissue transglutaminase antibodies in liver cirrhosis are common and method-dependent. *Clin Chim Acta* 2005; **356**: 102–9.

19 Almogren A, Kerr MA. Irreversible aggregation of the Fc fragment derived from polymeric but not monomeric serum IgA1-mediated disease. *Mol Immunol* 2008; **45** (1): 87–94.

20 McGowan KE, Lyon ME, Butzner JD. Celiac disease and IgA deficiency: complications of serological testing approaches encountered in the clinic. *Clin Chem* 2008; **54** (7): 1203–9.

21 Murdock AM, Johnston SD. Diagnostic criteria for coeliac disease: time for change? *Eur J Gastroenterol Hepatol* 2005; **17** (1): 41–3.

22 Rangnekar AS, Chey WD. Review: tissue transglutaminase test is almost as accurate as endomysial antibody test for diagnosis of coeliac disease. *Evid Based Med* 2007; **12** (1): 24.

23 Wessa P. Pearson correlation (v1.0.3). Free Statistics Software (v1.1.23-r3), 2008.

24 Sugai E, Vazquez H, Nachman F *et al*. Accuracy of testing for antibodies to synthetic gliadin-related peptides in celiac disease. *Clin Gastroenterol Hepatol* 2006; **4**: 1112–7.

25 Kumar PJ. European and North American populations should be screened for coeliac disease. *Gut* 2007; **52**: 170–2.

26 Grose RH, Cummins AG, Thompson FM. Deficiency of invariant natural killer T cells in coeliac disease. *Gut* 2007; **56**: 790–5.

27 Marignani M, Angeletti S, Morini S. Osteoporosis and liver disease: additional reasons for coeliac disease screening. *Gut* 2003; **52**: 452.

28 Selby W. Diagnostic tests: gluten enteropathy. *Australian Prescriber* 2001; **24**: 38–40.

29 Hobbs JR. Further aspects of human immunoglobulin A deficiency. *Ann Clin Biochem* 2007; **44**: 495–7.

30 Salmi TT, Collin P, Korponay-Szabó IR *et al*. Endomysial antibody-negative coeliac disease: clinical characteristics and intestinal autoantibody deposits. *Gut* 2006; **55**: 1746–53.

31 Kaukinen K, Peraaho M, Collin P *et al*. Small-bowel mucosal transglutaminase 2-specific IgA deposits in coeliac disease without villous atrophy: a prospective and randomized clinical study. *Scand J Gastroenterol* 2005; **40**: 564–72.

32 Korponay-Szabó IR, Halttunen T, Szalai Z *et al*. *In vivo* targeting of intestinal and extraintestinal transglutaminase 2 by coeliac autoantibodies. *Gut* 2004; **53**: 641–8.

33 Lundin KEA, Sollid LM. Gliadin peptide-specific intestinal T cells in coeliac disease. *Gut* 2007; **52**: 162–8.

34 Verkasalo MA, Raitakari OT, Viikari J *et al*. Undiagnosed silent coeliac disease: a risk for underachievement? *Scand J Gastroenterol* 2005; **40**: 1407–12.