Evidence supporting safe diagnosis of coeliac disease in children with antitissue transglutaminase titre ≥5 times upper limit of normal

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ABSTRACT
Objective European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) guidelines on coeliac disease (CD) recommend that children who have IgA-based antitissue transglutaminase (TGA-IgA) titre ≥10× upper limit of normal (ULN) and positive antiendomysial antibody, can be reliably diagnosed with CD via the no-biopsy pathway. The aim of this study was to examine the relationship between TGA-IgA ≥5×ULN and histologically confirmed diagnosis of CD.

Methods Data including TGA-IgA levels at upper gastrointestinal endoscopy and histological findings from children diagnosed with CD following endoscopy from 2006 to 2021 were analysed. CD was confirmed by Marsh-Oberhuber histological grading 2 to 3c. Statistical analysis was performed using χ² analysis (p<0.05= significant).

Results 722 of 758 children had histological confirmation of CD. 457 children had TGA-IgA ≥5×ULN and 455 (99.5%) of these had histological confirmation for CD; the two that did not had eventual diagnosis of CD based on clinicopathological features. 114 of 457 had between TGA-IgA ≥5×ULN and <10×ULN, all had confirmed CD. The likelihood of a positive biopsy with TGA-IgA ≥5×ULN (455/457) compared with TGA-IgA <5×ULN (267/301) has strong statistical significance (p<0.00001). The optimal TGA-IgA cut-off from receiver operating characteristic curve analysis was determined to be below 5×ULN for the two assays used.

Conclusion 99.5% of children with TGA-IgA ≥5×ULN had histological confirmation of CD; suggesting that CD diagnosis can be made securely in children with TGA-IgA ≥5×ULN. If other studies confirm this finding, there is a case to be made to modify the ESPGHAN guidelines to a lower threshold of TGA-IgA for serological diagnosis of CD.

INTRODUCTION
Coeliac disease (CD) is a systemic autoimmune condition triggered by ingestion of dietary gluten peptides found in wheat, rye and barley in genetically susceptible individuals.1 Various epidemiological studies have shown that prevalence of CD is approximately 1% in most populations.1-3 Initial serological screening involves measuring immunglobulin-A (IgA) level and IgA-based antitissue transglutaminase (TGA-IgA) titre.2 Once the diagnosis is confirmed, a lifelong gluten-free diet (GFD) is needed with regular monitoring and support.1 2

What is already known on this topic?
- Serological diagnosis of coeliac disease (CD) is highly sensitive, specific, less invasive and more economical than biopsy-based pathway.
- No-biopsy pathway (NBP) for diagnosing CD in children with antitissue transglutaminase (TGA-IgA) titre ≥10× upper limit of normal (ULN) has now become established clinical practice.

What this study adds?
- In our centre, a lower threshold of TGA-IgA ≥5×ULN can be used to reliably diagnose CD in children via the NBP.

How this study might affect research, practice or policy?
- Additional studies testing the optimal threshold of TGA-IgA to diagnose CD using different assays in different populations would be of value.
- Evolving evidence suggests that the European Society for Paediatric Gastroenterology Hepatology and Nutrition guidelines could be modified to propose that children with TGA-IgA ≥5×ULN can be diagnosed as having CD via NBP without need for endoscopy and biopsy.

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main criteria for making a diagnosis of CD via NBP. The presence of symptoms and HLA-DQ2/DQ8 testing are no longer obligatory for making a NBP diagnosis of CD for children with TGA-IgA ≥10×ULN.

Small bowel biopsies are still mandatory to make a definitive diagnosis in patients who do not fulfil the above criteria. This includes children with suspected CD who have IgA deficiency and risk a false negative diagnosis of CD, and those whose TGA-IgA is <10×ULN.

STUDY BACKGROUND
Since implementation of the NBP in 2013 in our centre, we have observed a reduction in upper gastrointestinal endoscopy (UGIE) examination for diagnosis of CD by 50%. This has contributed to significant reduction in the burden on endoscopy services and cost savings (approximately £1275 per patient) for the health service. When given the choice for diagnostic pathways, most families in our region have preferred NBP against an endoscopic biopsy.

The COVID-19 pandemic has caused an unexpected and unprecedented challenge for health services including endoscopy services. A recent survey carried out among paediatric gastroenterologists in 145 institutions across Europe and North America found that 90% of institutions postponed elective endoscopies.

We felt it was timely to study the concordance of a lower threshold of TGA-IgA titre with histological diagnosis of CD.

Aims and objectives
The objectives of this study were:
1. To examine the relation of histological confirmation of CD diagnosis with TGA-IgA ≥5×ULN in a large cohort in a single tertiary specialist centre.
2. To compare any differences between symptomatic and asymptomatic groups of children if diagnosis of CD is based on TGA-IgA ≥5×ULN.

METHODS
Our centre is located in the South West of England and is the single regional paediatric gastroenterology centre serving a total population of 6 million. It receives paediatric gastrointestinal referrals from 10 secondary care hospitals including diagnostic UGIEs for CD and direct referrals for suspected CD from general practitioners based in the local area. Children suspected of having CD have screening serology tests done in secondary care centres. The NBP based on 2012 ESPGHAN guideline was adopted in our unit in 2013. Children who had an elevated TGA-IgA below the threshold of 10×ULN were referred for diagnostic endoscopy and were advised to remain on a gluten containing diet. TGA-IgA positivity and subsequent histological findings for CD diagnosis in patients who do not fulfil the above criteria. This was used to compare the rate of histologically confirmed CD for TGA-IgA ≥5×ULN and <5×ULN; p<0.05 was considered significant. Positive and negative predictive values (PPV and NPV, respectively) were calculated as described in Altman et al using the ‘yardstick’ R package (Vx0.0.7) (https://CRAN.R-project.org/package=yardstick). The receiver operating characteristic (ROC) curve was plotted with different cut-offs of the TGA-IgA test using the ROCit R package (V2.1.1) (https://CRAN.R-project.org/package=ROCit) and from this, an optimal cut-off was determined by the Youden method. R statistical software (V4.0.3) was used for this analysis.

This project was registered with the clinical audit department as a service review. No patient identifiable data were collected and ethical approval was not considered necessary.

RESULTS
A total of 1001 patients attended for UGIE for investigation of CD over the 15-year study period. A total of 243 patients were excluded either because they were IgA deficient or did not have a TGA-IgA taken on the day of diagnostic endoscopy. A total of 758 were included in the study. Of the 758 patients, 588 were from the cohort (2006–2017) where the ULN for the TGA-IgA was <10IU/mL (group 1) and 170/758 were from the cohort (2018–2021) where the ULN for the TGA-IgA was <4IU/mL (group 2). A total of 168 of the 758 children were asymptomatic at presentation. Figure 1 details the clinical flowcharts showing TGA-IgA positivity and subsequent histological findings for patients with suspected CD.

Table 1 highlights the demographics for the study group including age, sex and ethnicity. Figure 2 depicts the various manifestations at presentation that led to the diagnosis of CD. The majority had either gastrointestinal symptoms, EIM or a mixture of these (78%).

Of the 758 patients, 722 received a histological confirmation of CD (MO-HG 2–3c). A total of 457 (60.3%) patients had TGA-IgA ≥5×ULN and 453 of these (99.5%) had positive histopathology for CD (MO-HG 2–3c). Twenty-five per cent of these children (114/457) had TGA-IgA between ≥5×ULN and <10×ULN and all 114 had histological confirmation for CD (MO-HG 2–3c). Two patients with TGA-IgA ≥10×ULN, had non-diagnostic biopsies (MO-HG 1); they were both symptomatic on the day of UGIE. Pathologist commented that the biopsies were poorly oriented despite further cuts. At the clinico/pathology meeting, the two cases were discussed and a provisional diagnosis of CD was agreed. They subsequently had
a good response to GFD with normalisation of TGA-IgA and hence were given a final diagnosis of CD.

Out of 301 patients who had TGA-IgA <5×ULN, 34 (11.3%) did not have histological confirmation (MO-HG 0–1) of CD. A TGA-IgA ≥5×ULN had significantly greater likelihood of a positive biopsy (455/457 (including 114 with TGA-IgA <10×ULN and ≥5×ULN all of whom had a confirmed histological diagnosis of CD)) than TGA-IgA <5×ULN (267/301) (χ² p<0.00001).

Group 1 contained 588 patients. The ROC curve showed an optimal cut-off of 24.5 IU/mL that is, 2.5×ULN (specificity=0.82, sensitivity=0.85) with an area under the curve of 0.8831 (figure 3). The PPV and NPV for TGA-IgA levels ≥5×ULN (≥50 IU/mL) were 0.997 and 0.0788, respectively. In group 2 containing 170 patients, the ROC curve showed an optimal cut-off of 19.5 IU/mL, that is, 4.9×ULN (specificity=0.95, sensitivity=0.47) with an area under the curve of 0.7219 (figure 4). The PPV and NPV for TGA-IgA levels ≥5×ULN (≥20 IU/mL) were 0.986 and 0.186, respectively.

IgA anti-endomysial antibodies (EMA-IgA) results were available in 481/722 (66.6%). EMA-IgA results were available in 233/457 patients with CD with TGA-IgA ≥5×ULN and was positive in 221 cases (94.9%). For patients with TGA-IgA <5×ULN, 248/299 had EMA-IgA results available and was positive in 236 (95.2%). EMA-IgA results were not significantly different between the two groups (p=0.95).

Out of 168 asymptomatic patients, 158 received a histological confirmation of CD. Ten children did not have CD, all had a TGA-IgA <5×ULN. A total of 115 of the 158 asymptomatic children confirmed as CD had a TGA-IgA ≥5×ULN. Table 2

**Table 1** Demographic and characteristics of patients confirmed with coeliac disease (n=722)

| Patient characteristic | TGA-IgA <5×ULN (n=267) | TGA-IgA ≥5×ULN (n=455) |
|------------------------|------------------------|------------------------|
| Sex                    | Male 92                | 170                    |
|                        | Female 175             | 285                    |
| Age (in years)         | Mean age 9.80          | 8.02                   |
|                        | Median age 10          | 8                      |
|                        | Range of ages 1–17     | 0–17                   |
| Ethnicity              | Caucasian 257          | 438                    |
|                        | Non-Caucasian (includes children from Asian, African and Afro-Caribbean descent) 10 | 17 |

TGA-IgA, IgA-based antitissue transglutaminase antibody.
lists the reasons that prompted serological screening in asymptomatic children; the most common reasons were:
1. A pre-existing diagnosis of Ty-1 DM in 60 of 158 (38%),
2. A positive family history of CD in first-degree relatives in 58 of 158 children (36.7%).

DISCUSSION
This 15-year study from our centre found that 455 of 457 (99.5%) children with TGA-IgA ≥5×ULN had clear histological confirmation of CD. Moreover, all 114 of 457 with TGA-IgA <10×ULN and ≥5×ULN children (irrespective of whether they were symptomatic/asymptomatic) received histological confirmation of CD. The optimal cut-off derived from the two ROC analyses of 2.5×ULN and 4.9×ULN are less than the current ESPGHAN guidance of 10-times the ULN for TGA-IgA titres. The high PPV provides evidence that TGA≥5×ULN is associated with small bowel enteropathy consistent with a diagnosis of CD in children and suggests that a lower cut-off of ≥5×ULN may be as reliable as TGA-IgA ≥10×ULN. A significant (11.3% (34/301)) number of patients with TGA-IgA <5×ULN did not have histological features of CD; hence, small bowel biopsies should remain essential for definitive diagnosis in these children.

If the threshold for NBP was reduced to TGA-IgA ≥5×ULN in our region, a further 15.8% of patients (114/722) may not require UGIE. This is timely data due to additional pressure on endoscopic services due to the COVID-19 pandemic.

The results of this study are similar to two other published studies.11 12 A study from India over a 14-month period involving 142 children aged ≤12 years reported that a TGA-IgA titre of >4×ULN, had 95.4% sensitivity and 98% specificity for a confirmed histological diagnosis of CD.11 An Italian study of 84 children with CD whose TGA-IgA titres were between 5×ULN and 10×ULN, found histological changes compatible with CD in 78/84 (92.8%); the authors concluded that during the COVID-19 outbreak, a temporary reduction of the TGA-IgA threshold for biopsy-sparing approach was feasible.12 Results from these and our study support the view that the threshold for NBP could be safely lowered to ≥5×ULN. However, further studies including a range of different assays may be required before this concept can be fully established in wider clinical practice guidelines.

Testing for EMA-IgA was not compulsory for the ESPGHAN biopsy-based diagnostic pathway and our laboratory did not offer it automatically until 2013. A separate request was required prior to this period (2006–2013 data set). Hence, EMA-IgA results available in only 481 cases were analysed. EMA-IgA results were not different between patients above and below the threshold of TGA-IgA ≥5×ULN (p=0.95) and did not positively contribute to the overall diagnostic pathway. EMA-IgA data were not available for 33% of patients, as it was not essential in serological workup for histological diagnosis.

We have presented one of the largest datasets for this important objective. This was not a prospectively established research study, so there were two different TGA-IgA assays used reflecting the

### Table 2 Reasons for initial screening of asymptomatic children with confirmed diagnosis of coeliac disease (n=158)

| Reason for screening | TGA-IgA <5×ULN (n=43) | TGA-IgA ≥5×ULN (n=115) |
|----------------------|------------------------|------------------------|
| Screened for groups at high risk of developing CD (n=140) | | |
| Type 1 diabetes | 6 | 54 |
| Family history of CD | 20 | 38 |
| Hypothyroidism | 1 | 3 |
| Other autoimmune disorders | 4 | 1 |
| Trisomy 21 | 3 | 7 |
| Turner syndrome | 1 | 1 |
| Cystic fibrosis | 0 | 1 |
| Identified incidentally while investigating other conditions (n=18) | | |
| Conditions included: family history of CD in second degree relatives (eg, grandparents or cousins), food allergies, unexplained proteinuria, EAT study, eating disorder | 8 | 10 |

CD, coeliac disease; EAT, enquiring about tolerance; TGA-IgA, IgA based antitissue transglutaminase antibody; ULN, upper limit of normal.
change in clinical practice in a real world setting. This does provide a limitation to the ROC analysis but it is clinically useful to demonstrate that the lower threshold of TGA-IgA ≥5×ULN works well for at least two different TGA-IgA assays. There were a group of children who underwent endoscopy despite a TGA-IgA ≥10×ULN. A proportion of these were families who chose to have endoscopy to confirm the diagnosis or were asymptomatic at presentation and were diagnosed prior to the publication of the 2020 ESPGHAN guidelines. However, the majority had a TGA-IgA below 10×ULN in the regional hospital. This may be due to variation in assay performance which individual centres need to be mindful of. Over the study period of 15 years, there have been a number of different pathologists reporting paediatric gastrointestinal biopsies. These specimens have not been reviewed by a single blinded pathologist, as all the cases over the years were discussed in a multidisciplinary meeting containing at least one pathologist and paediatric gastroenterologists.

**CONCLUSION**

This large single-centre experience illustrates that the diagnosis of CD can be made securely in children with TGA-IgA ≥5×ULN for two different TGA-IgA assays without the need for UGIE. Considering the challenges posed by the COVID-19 pandemic, individual centres and national societies may wish to review their own data to see if a threshold of TGA-IgA ≥5×ULN could be used safely to diagnose CD without biopsy.

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