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The role of a point of care test for the diagnosis and management of coeliac disease

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Abstract

Coeliac disease is a systemic autoimmune disease associated with gastrointestinal and extra-gastrointestinal symptoms, triggered by gluten in genetically susceptible individuals. It affects 1% of the general population (1, 2), although 75% remain undiagnosed (3). Delayed diagnosis can lead to a poor quality of life and complications (3). The under-detection could be due to non-specific symptoms and under-utilisation of serological testing (4). Several point of care tests for coeliac disease have been developed in the past decade, which may potentially help to improve case detection.

A few recent studies have shown that Simtomax, a point of care test detecting IgA-/IgG-deamidated gliadin peptide antibodies (IgA/IgG-DGP), appeared to have comparable sensitivities to conventional serology. However, further studies are required to validate the diagnostic performance of Simtomax.

The null hypothesis of my thesis is that a point of care test has no role in the diagnosis and management of coeliac disease. We aimed to evaluate the sensitivities, utility and cost effectiveness of the point of care test, Simtomax, in various domains:

Study 1: To assess the role of Simtomax as a primary care case finding tool for coeliac disease in high risk individuals in community pharmacies.

Study 2: To demonstrate the diagnostic accuracy of Simtomax in secondary care, in patients referred with gastrointestinal symptoms or self-reported gluten sensitivity.

Study 3: To establish the diagnostic accuracy of Simtomax and its cost effectiveness of coeliac testing in patients with iron deficiency anaemia in the endoscopy setting.

Study 4: To investigate whether Simtomax is a reliable surrogate marker for predicting histological remission in patients with known coeliac disease on a gluten free diet.

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List of abbreviations

AGA- Anti-gliadin antibodies

APC- Antigen presenting cell

AUC- Area under the curve

BSG- British Society of Gastroenterology

CD- Coeliac disease

CD4- Cluster of differentiation 4

CI- Confidence interval

DGP - Deamidated gliadin peptide antibodies

EATL – Enteropathy Associated T-cell Lymphoma

ELISA - Enzyme-Linked Immunosorbent Assay

EMA – Endomysial antibodies

ESPGHAN- European Society for Paediatric Gastroenterology Hepatology and Nutrition

HLA – Human Leucocyte Antigen

HP- Helicobacter pylori

HR- Hazard ratio

HRG- Health Resource Group

IEL- Intraepithelial lymphocytes

IgA- Immunoglobulin A

IL- Interleukin

MMP- Matrix metalloproteinase

NCGS- Non-coeliac gluten sensitivity

NHS- National health service

NKG2D- Natural killer group 2 member

NICE- National institute of health and care excellence

NPV – Negative predictive value

OR- Odds ratio

PPV – Positive predictive value

RCD- Refractory coeliac disease

ROC curve- Receiver operating characteristic curve

SD – Standard Deviation

TNF-α- Tumour necrosis factor-α

TTG – Tissue transglutaminase antibodies

Chapter 1: Overview of coeliac disease

1.1: Introduction to Coeliac Disease

1.1.1: The history of coeliac disease

Coeliac disease is a systemic autoimmune disease associated with gastrointestinal and extra-gastrointestinal symptoms, affecting 1% of the general population worldwide (1, 5). It is 1.5-2 times more prevalent in females (1, 3, 6). The disease is triggered by dietary gluten, a ubiquitous protein complex found in staple foods made of wheat, barley and rye in genetically susceptible individuals (7). Although this condition of gluten intolerance may appear to be a modern disease, it was in fact described since ancient times. Humans had been hunter gatherers since the beginning of humankind 2.5 million years ago, until wheat cultivation methods were first adopted in Gobleki Tepe in south eastern Turkey, known as the Fertile Crescent, from 10th century CE. This Neolithic agricultural revolution transformed humankind's way of life from hunter gatherers to securing a stable food supply. Consequently, wheat cultivation generated antigens that were previously unknown to humans, and those who were unable to adapt would develop coeliac disease.

During the 2nd century CE a Greek physician named Aretaeus of Cappadocia, who lived in eastern Turkey, identified this malabsorptive syndrome of steatorrhea, weight loss and pallor, and named it 'koiliakos', meaning abdominal. Centuries later in 1887, Samuel Gee, a leading English paediatrician, provided the first comprehensive modern description of this chronic malabsorptive disorder that could be treated by dietetic measures, for which he named as coeliac disease, concluding 'if the patient can be cured at all, it must be by means of diet', although the causative agent was not known at the time.

In 1924, the famous banana diet was championed by an American physician, Sidney Haas (8). The diet excluded other carbohydrates such as bread, cereals, crackers and potatoes, which benefited many children for decades based on eliminating wheat grains. A significant discovery was made after the second World War in 1950, confirming the Dutch paediatrician, William Dicke's previous clinical observation of wheat being the culprit of coeliac disease. Dicke recognised that the shortage of bread towards the end of World War two, the so called 'winter of starvation', resulted in clinical improvement in children who were fed other foods such as rice and maize. And when bread was airdropped in Holland, the children rapidly deteriorated again. The toxic effect of wheat was supported by objective measurements of growth curves and faecal fat quantification related to wheat exposure. His subsequent collaborative studies found that the gliadin component of wheat was responsible for the fat malabsorption in coeliac patients (9).

A major breakthrough in coeliac disease pathology was made in 1954 when an English physician John Paulley discovered the characteristic small bowel mucosal flattening in patients with steatorrhoea through intestinal sampling during laparotomies. Small intestinal sampling was subsequently made less invasive by the invention of a flexible biopsy tube which took duodenal biopsies per orally with fluoroscopic guidance in 1956. This was later superseded by the Crosby capsule in 1957, which allowed jejunal biopsy sampling by suctioning the mucosa and triggering a spring activated knife to obtain the biopsy. This technique was widely used until the advent of fibreoptic endoscopes in the 1990's.

1.1.2: Clinical presentation

The presenting features of coeliac disease are variable and can be non-specific. According to the Oslo definitions, the presentation of coeliac disease can be categorised into two main groups- 'classical' and 'non classical' (10). Historically, coeliac disease was considered a rare disease, featuring 'classical' symptoms of malabsorption including chronic diarrhoea, weight loss and failure to thrive (11). It is important to note that the term 'classical coeliac disease' does not imply that it is the commoner form of presentation. In fact, since the 1980s, there has been a gradual recognition that coeliac disease often presents insidiously, with the majority of patients presenting with 'non classical' features such as irritable bowel type symptoms, anaemia, chronic fatigue, change in bowel habit, abdominal pain, osteoporosis and neurological symptoms (11-15). Epidemiological studies also showed that coeliac disease affects a far more diverse population worldwide among different races than previously recognised (16-19).

1.1.3: Epidemiology

Epidemiological studies have demonstrated that the true prevalence of coeliac disease is approximately 1% in Western countries (1, 5), with a female preponderance of 1.5-2 fold compared to males (1, 3, 6). The incidence of coeliac disease has been on the rise, through the combination of the advent of serological testing, heightened awareness of coeliac disease and a genuine increase in incidence. A recent Norwegian study found a three-fold increase in the incidence of biopsy proven coeliac disease in children from 2000 to 2010 (20). A similar trend has also been observed in adults where a Finnish group found the prevalence to have doubled between 1978-80 and 2000-01 (21). The authors concluded that there was a true increase in the

incidence of coeliac disease, as the prevalence of unrecognised coeliac disease increased proportionately with the recognised cases over the two decades. If the increased rate was purely due to a heightened awareness, then one would expect a drop in the proportion of undetected cases.

Apart from improved case detection from raised awareness, there are other factors that are postulated to cause the true rise in incidence. The increasing incidence of coeliac disease is parallel to the steady rise of other autoimmune diseases and allergies, with growing evidence to support the hygiene theory as one of the possible contributing factors to this phenomenon (22).

Furthermore, gluten is ubiquitous in the Western diet and processed foods, which may also play a part in the growing incidence of coeliac disease. Mounting evidence revealed that the prevalence of coeliac disease in Asia is climbing with an increasingly Westernised diet. The seroprevalence of coeliac disease was found to be 1.6% in Asia (Iran, Turkey, Israel and India) (23). Similar figures have been observed in population screening studies in Argentina (0.95% biopsy proven) (24), Malaysia (1.25% seroprevalence) (25) and Saudi Arabia (1% biopsy proven) (26).

1.1.4: Genetics

Genetic predisposition plays a key role in the pathogenesis of coeliac disease. Virtually all patients with coeliac disease carry the human leucocyte antigen (HLA) class II genes: the HLA-DQ2 haplotype is expressed in 95% of patients, and the HLA-DQ8 haplotype in the remaining 5%. The absence of these genotypes therefore effectively rules out coeliac disease (27). These HLA class II genes are not exclusive to patients with coeliac disease however, as they are also expressed in 30% of the general population where coeliac disease is prevalent. Interestingly, only 2-5% of individuals carrying these class II major histocompatibility complex molecules develop coeliac disease, implicating that environmental factors also play a part (28).

1.1.5: Pathogenesis

Gliadin is a glycoprotein extract from gluten that is unusually proline rich, a residue that is very resistant to degradation by luminal and brush border peptidase (29). Long fragments of gliadin trigger the release of zonulin, a physiological modulator of tight junctions, allowing gliadin to cross the intestinal epithelial barrier through increased gut permeability (30). (Figure 1)

Adaptive immune response

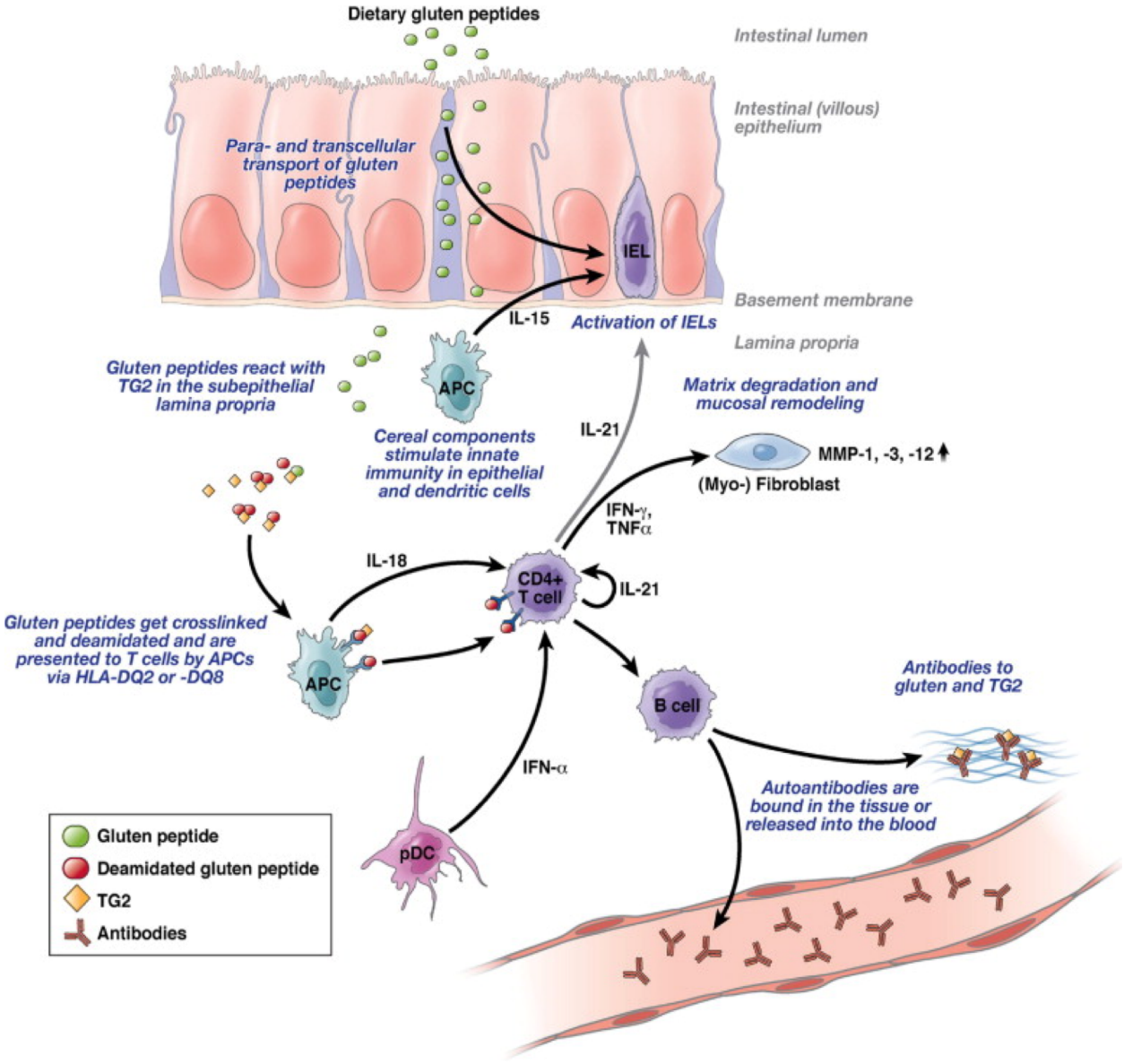
Once in the lamina propria, gliadin is deamidated to negatively charged glutamic acid by tissue transglutaminase (TTG), an enzyme normally involved in collagen cross-linking and tissue remodelling. The deamidated gliadin peptides (DGP) have a high affinity to bind to HLA-DQ2 or HLA-DQ8 molecules expressed on the antigen presenting cells (APC). This triggers an adaptive immune response, where the APC interact with gliadin specific cluster of differentiation 4 (CD4)

T-helper 1 cells to produce inflammatory cytokines (31). Inflammatory cytokines such as interferon (IFN)-γ induce the activation and release of matrix metalloproteinases (MMP) by myofibroblasts, resulting in mucosal remodelling and villous atrophy. On the other hand, T-helper 2 cells drive the clonal expansion of B cells to produce anti-gliadin (AGA) and anti-TTG (32). (Figure 1)

Innate immune response

Gliadin also triggers an innate immune response involving an increased production of pro-inflammatory cytokines, particularly interleukin (IL)-15. IL-15 upregulates the natural-killer group 2 member D (NKG2D) receptors on intraepithelial lymphocytes (IEL) and its epithelial ligand, inducing epithelial apoptosis (28). Intriguingly, α-gliadin peptides unrecognised by intestinal CD4 T cells can upregulate IL-15 production, suggesting that the innate response in the epithelium may occur autonomously, as seen in patients with latent coeliac disease prior to the occurrence of villous atrophy (33). (Figure 1)

Figure 1: The pathogenesis of coeliac disease. Increased gut permeability allows gluten peptides (gliadin) to cross the intestinal epithelial barrier and enter the lamina propria. Gliadin is deamidated by TTG which enhances its binding to HLA DQ2 and HLA DQ8 molecules that are presented on APC. This complex is recognised by CD4 T helper 1 cells which produce inflammatory cytokines such as IFN-γ, inducing mucosal remodelling and villous atrophy by MMP released by myofibroblasts. T-helper 2 cells drive the clonal expansion of B cells to produce AGA and anti-tissue transglutaminase (TG2 in the figure). Gliadin also stimulates an innate immune response involving an increased production of pro-inflammatory cytokines such as IL-15 in epithelial and dendritic cells, activating intraepithelial lymphocytes and ultimately causing epithelial apoptosis.



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1.1.6: Complications

Patients living with undiagnosed coeliac disease often suffer from a significantly impaired quality of life compared to those who have been diagnosed and treated (health outcome measure EQ-5D 0.56 versus 0.84) (34). Anaemia and nutritional deficiencies in iron, vitamin B12, vitamin D, folate, and calcium are common at diagnosis (35). The prevalence of metabolic bone disease and premature osteoporosis is more than twice than that of the general population (36). Although there is a moderate increased risk of fractures in patients with coeliac disease (hazard ratio [HR] 1.31 for any fractures) compared to the general population, the absolute fracture risk is small (3.19 per 1000 person-years) (37). Extra-gastrointestinal complications such as subfertility, adverse foetal outcomes (38, 39) and neuropsychiatric symptoms (e.g. headaches, depression) (40, 41) are not uncommon.

A small proportion of patients will develop non-responsive coeliac disease, defined as failure of symptomatic or histological improvement following presumed adherence to a gluten free diet (primary non-response if no response after 12 months of a gluten free diet; and secondary non-response if symptoms relapse after initial response to a gluten free diet). The commonest cause for non-responsiveness is gluten exposure, whether inadvertently or not (42), due to the restrictiveness of a gluten free diet and the ubiquity of gluten in processed foods. Dietary adherence has been reported to be 36%-96%, and is influenced by a variety of cognitive, emotional and socio-cultural factors (43, 44). Other causes for persistent symptoms should be ruled out, such as microscopic colitis, pancreatic insufficiency and small bowel bacterial overgrowth.

In patients with persistent villous atrophy despite a confirmed strict gluten free diet by a coeliac specialist dietitian, a diagnosis of refractory coeliac disease (RCD) may be made after exclusion of other concomitant small intestinal conditions causing villous atrophy (e.g. small bowel bacterial overgrowth, giardiasis, tuberculosis, human immunodeficiency virus and Whipple's disease). RCD is a rare complication defined by persistent or recurrent malabsorptive symptoms and villous atrophy despite strict adherence to a gluten free diet for at least 6-12 months, in the absence of other causes of non-responsive treated coeliac disease (45, 46). The true incidence of RCD is unknown due to its rarity. Within the coeliac cohort, the prevalence has been reported to be 0.7% in a UK study, and cumulative incidence of 1.47% in a US study (47, 48). Symptoms are often severe and patients often require pharmacological therapies in addition to a strict gluten free diet.

RCD can be divided into two types based on the presence or absence of aberrant IELs in the small bowel mucosa. Type 1 RCD is clinically and histologically indistinguishable from uncomplicated active coeliac disease. It could represent patients who are super sensitive to even trace levels of gluten, or those with a slow response to gluten withdrawal. Type 1 RCD generally have a more benign course compared to type 2, although the rate of complications and mortality is still much higher than uncomplicated coeliac disease (45, 49). In type 2 RCD, severe complications such as ulcerative jejunitis and enteropathy associated T cell lymphoma (EATL) can develop. There is a marked difference in the five year survival rate between type 1 and type 2 RCD (96% vs 58%), which is largely explained by the high frequency of development of EATL in patients with type 2 RCD (52%) within 4-6 years of diagnosis (49, 50). EATL carries a grave prognosis, with a five year

survival of only 11% despite aggressive treatment (51-53).

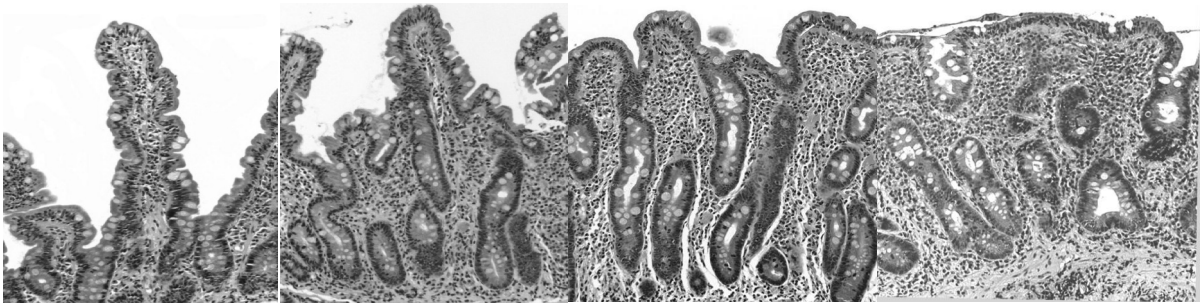
Due to the rarity of RCD, evidence for therapeutic interventions for RCD are based on case reports and open label observational studies. Immunosuppressants such as prednisolone, budesonide and/or azathioprine have been shown to be effective in inducing and maintaining clinical and histological remission in most type 1 RCD patients (54-56). In contrast, there is no effective treatment for type 2 RCD. The only drug that was shown to induce clinical and histological improvement with a significant decrease in the number of aberrant clonal IELs is an antimetabolite, cladribine. Nevertheless, 41% of patients still died of EATL despite cladribine therapy (57). The risk of lymphoproliferative malignancies such as EATL has been shown to be increased in patients with persistent villous atrophy compared to those with mucosal healing (HR 2.26) (58). Fortunately, EATL is a rare complication of coeliac disease, as demonstrated in a nationwide Swedish study showing an absolute rate of lymphoproliferative malignancies in coeliac disease of 70.3 per 100,000 person-years (59).

1.1.7: Diagnosis

The diagnosis of coeliac disease has evolved considerably over the last few decades as our understanding of its pathophysiology came to light. According to the current British Society of Gastroenterology (BSG) (35) and National Institute for Health and Care Excellence (NICE) (60) guidelines, a diagnosis of coeliac disease is made based on a positive coeliac serology with Marsh

grade 3 histology, consisting of raised IELs (>25 per 100 enterocytes), crypt hypertrophy, and villous atrophy (Figure 2).

Figure 2: Duodenal histology findings in coeliac disease. From left to right- Marsh 1: Normal villous architecture with increased intraepithelial lymphocytes (IELs); Marsh 3a: Increased IELs, crypt hyperplasia and mild villous atrophy; Marsh 3b: Increased IELs, crypt hyperplasia and moderate villous atrophy; Marsh 3c: Increased IELs, crypt hyperplasia and total villous atrophy. Stained with haematoxylin and eosin, ×100. Marsh 2 histology (Normal villous architecture with increased IELs and crypt hyperplasia) is not illustrated here.



Historically, case detection was an arduous three phase process based on the presence of 'classical symptoms' of malabsorption, with non-specific tests assessing for small bowel absorption, such as faecal fat, and a confirmatory jejunal biopsy showing villous atrophy. Because there are other causes for villous atrophy apart from coeliac disease, a relationship between gluten and the small bowel mucosa had to be demonstrated. This consisted of phase two showing symptom resolution on a gluten free diet and a second biopsy showing mucosal healing. A third biopsy was performed in phase three after a period of gluten challenge to demonstrate a recurrence of symptoms and mucosal damage on histology.

The advent of serological testing revolutionised the diagnostic methods for coeliac disease (61). A Swiss immunologist Berger detected antigliadin antibodies (AGA) in 1964. However, AGA has a low specificity giving a high number of false positives (62), therefore it is no longer recommended for the diagnosis of coeliac disease. In 1983, a Polish dermatologist Chorzelski discovered endomysial antibodies (EMA) and dermatitis herpetiformis in children with coeliac disease. Although EMA is highly specific, its sensitivity is low. Moreover, EMA testing is labour intensive, costly and its interpretation is subjective. The test is performed by Indirect immunofluorescence using rhesus monkey oesophagus as the substrate, with the endomysium of the smooth muscle bundles serving as the antigen. The sections are incubated with dilutions of the patient's sera and fluorescein conjugated anti-IgA. The slides are then screened and read manually using a fluorescent microscope, and fluorescent activity is graded against control slides. The complexity and subjectivity of EMA testing encouraged efforts to develop TTG testing, which uses enzyme linked immunosorbent assay (ELISA) technique that further transformed the diagnostic process (63). In principle, ELISA involves adding diluted patient sera to microwells that are pre-coated with recombinant human TTG antigen. Autoantibodies recognise the TTG antigen and bind to it, giving a blue reaction for positivity after a substrate is added. The optical density is read at 450nm using an automated and calibrated microplate reader, giving a TTG titre that corresponds to the intensity of the colour. The introduction of TTG has largely replaced AGA due to its superior sensitivity and specificity (64).

The BSG and NICE guidelines recommend screening high risk adults with TTG as a first line test in view of its excellent sensitivity, followed by EMA for those with a positive TTG (useful in cases

where TTG has a mildly raised titre which may be a false positive if EMA is negative), and a confirmatory duodenal biopsy for those with a positive EMA. On the other hand, the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) guidelines recommend that paediatric patients no longer require biopsies to confirm the diagnosis of coeliac disease if they have TTG levels 10 times over the upper limit of normal, positive EMA and the presence of HLA-DQ2 or HLA-DQ8 genotype. This was based on a few studies showing good predictability of coeliac disease at such high TTG titres (65).

1.1.8: Management

At present, a gluten free diet for life is the only effective and available treatment for coeliac disease. Small bowel absorption should be assessed by testing for full blood count, ferritin, folate and B12. Liver function tests should also be performed as coeliac disease can be associated with deranged liver enzymes. A recent study showed that liver function was deranged in 40.6% patients with coeliac disease at diagnosis, which normalised in 78% patients 1.5 years after gluten exclusion. Those without any improvement in liver function should be tested for any co-existing liver disease (66). Calcium, vitamin D and alkaline phosphatase should be monitored and supplemented accordingly. Those with additional risk factors for osteoporosis or age over 55 should have a baseline bone density scan at one year after commencement of a gluten free diet. Other co-existing autoimmune diseases should be screened for, such as autoimmune thyroid disease and type 1 diabetes. Functional hyposplenism is seen in patients with coeliac disease, which may lead to impaired immunity to encapsulated bacteria. Data showed a modest increase in pneumococcal sepsis in patients with coeliac disease compared to the general population (HR

2.6), therefore annual pneumococcal vaccination is recommended (67).

The goal of treatment is to relieve symptoms, achieve mucosal healing and prevent complications through a strict gluten free diet. Therefore, assessment of adherence and disease remission are key aspects of management (68). The assessment of adherence incorporates a combination of dietetic evaluation by a coeliac specialist dietitian, symptom assessment, serological testing and repeat duodenal biopsy to assess for mucosal healing. Monitoring of complications includes haematological and biochemical evaluation for anaemia and nutritional deficiencies, bone density scans for osteoporosis, and vaccinations against Pneumococcus for functional hyposplenism (35). Any suspicion of serious complications such as RCD, ulcerative jejunitis or EATL should be urgently investigated endoscopically with small intestinal biopsies and small bowel capsule endoscopy. Immunosuppressants and artificial nutritional support may be indicated in RCD.

Novel pharmacological therapies for coeliac disease are on the horizon, although only several have undergone phase 2 clinical trials. Gliadin is known to increase the secretion of a tight junction modulator, zonulin, which increases intestinal permeability. This facilitates the transport of gliadin into the lamina propria through the compromised epithelial barrier, thereby triggering an inflammatory process. Phase 2 randomised controlled trials on a zonulin inhibitor, larazotide acetate (AT-1001), have shown conflicting results regarding the preservation of intestinal epithelial integrity to gluten exposure, although patients in the larazotide group experienced less symptoms on a gluten challenge (69-71).

Another therapeutic option is detoxifying gluten by degradation of gluten peptides which are highly resistant to enzymatic proteolysis in the gut. Phase 2 randomised controlled trials have demonstrated that pre-treating gluten with bacterial endoproteases (ALV003 and AN-PEP) led to significantly less mucosal injury and T cell response (72-74).

A therapeutic vaccine designed to induce immunotolerance to gluten has been developed based on three frequently recognised immunogenic peptides (gliadin, hordein and secalin) identified in patients with coeliac disease. Regular administration of the vaccine in mouse models have shown a dose dependent decrease in T cell proliferation on gluten exposure. Phase 1 trials have shown that the Nexvax2 vaccine when given in gradual escalation of doses over 7-9 weeks (with dose intervals of once every 3-4 days) was safe and well tolerated without immune activation or serious adverse effects (75). Nexvax2 will now enter phase 2 trials to be studied as an adjunct to a gluten-free diet. There are a number of other therapeutic options being studied, but they are still in their infancy in preclinical phases. If the aforementioned therapies are proven to be efficacious, it is foreseeable that they will act as adjuncts to a gluten free diet perhaps in a subset of patients who have difficulty in inducing or maintaining remission, rather than as a replacement of a gluten free diet given the potentially high cost.

1.2: Point of Care Tests for Coeliac Disease

1.2.1: Why do we need a point of care test for coeliac disease?

The fact that 76% of patients with coeliac disease remain undiagnosed suggests that the current case finding strategies with serological testing may be inadequate. Despite national guidelines, it

has been revealed that coeliac serology was only performed in 31.8% of patients with symptoms suggestive of coeliac disease who were undergoing a gastroscopy with duodenal biopsies, implying that serological testing was underused (4). Conventional serological testing is a sensitive screening tool for coeliac disease, although it is not without drawbacks.

Serology testing requires venepuncture by a health care professional and can be difficult in children. It is performed in central laboratories, and results usually take several days to a week to become available. Moreover, EMA testing is labour intensive, time consuming and costly, and is also prone to subjective interpretation of results. Serological testing usually requires multiple healthcare visits and can lead to a delay in the patient undergoing a gastroscopy to obtain duodenal biopsies for confirmation of the diagnosis and initiation of treatment.

The advantages of point of care tests are manifold: point of care tests do not require venepuncture nor laboratory processing, thus operate at a relatively low cost compared to conventional serology. A cost benefit analysis showed that by replacing current serological testing with a point of care test, Simtomax, in patients with suspected coeliac disease in primary care, a potential cost saving of over £7 million per year could be made for the National Health Service (NHS) (76). As previously mentioned, serology results were only available in one third of patients with suspected coeliac disease who were attending for a gastroscopy (4). The lack of a serological result commits the endoscopist to taking duodenal biopsies routinely for patients with symptoms suggestive of coeliac disease, which is an expensive method for case detection. Most importantly, point of care tests can be performed in an ambulatory care setting with rapidly

available results, which could facilitate the clinician's immediate decision making to streamline the diagnostic process.

1.2.2: The commercially available point of care tests for coeliac disease

A point of care test, also known as a rapid test or near patient test, is a test that can be performed near the patient, providing rapid results without the need for laboratory processing. Point of care tests for coeliac disease have evolved considerably in the past two decades. One of the first point of care tests was a dot immunobinding assay strip detecting AGA, with results available in 30 minutes (77, 78). With the discovery of TTG being identified as an antigen in coeliac disease (31), several point of care tests detecting TTG have since been developed. Early point of care tests by dot blot immunoassay test strips (Figure 3) (79-81) have been superseded by more user friendly, commercially available point of care test kits using lateral flow immunochromatography, giving rapid results in 10 minutes (Figure 4). The commercially available POCTs are listed in table 1.

Figure 3: An early rapid test by dot blot immunoassay test strips.

A red blot at the control line denotes a correctly functioning test strip. A red blot at the test line indicates a positive TTG result. Test strips 1, 4, 6, 7 and 9 are positive samples, and 2, 3, 5, 8 and 10 are negative samples.

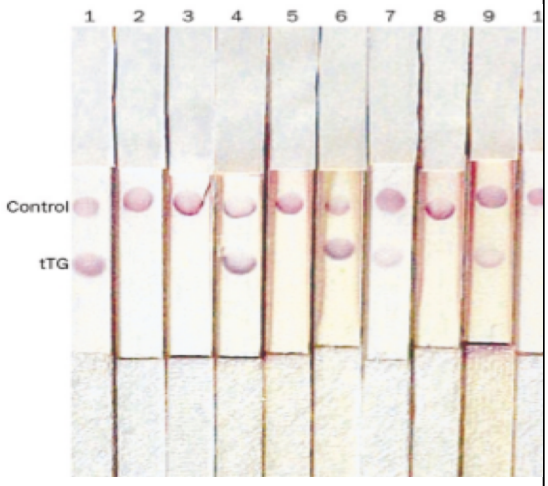


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Figure 4: A commercially available point of care test, using Simtomax as an example.

A drop of blood from a finger prick is drawn with a pipette (top left), which is then applied to the test device followed by 5 drops of the provided buffer solution (top right). (a) Results can be read within 10 minutes. (b) A red test line A indicates a positive test result, a red test line B indicates the presence of immunoglobulin A (IgA), and an inbuilt red control line CT ensures that the device is functioning correctly. (c) shows a negative Simtomax test.

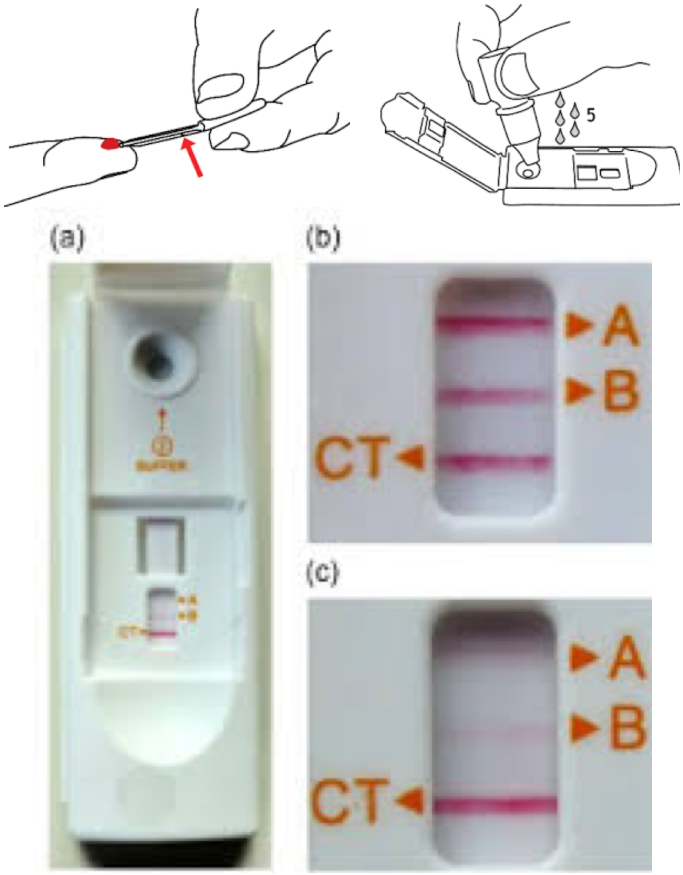

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Table 1: The commercially available POCTs.

POCT	Substrate	Antibody	Control line	Available in the UK	Available for purchase by the public or health care professionals (HCP only)
Biocard[A]	Whole blood	IgA TTG	Yes	Yes	Public and HCP
Stick CD1[B]	Serum	IgA/IgG TTG	No	No	HCP only
Stick CD2[B]	Serum	IgA TTG and AGA	No	No	HCP only
Simple CD1WB [B]	Whole blood	IgA/IgG/IgM TTG	Yes	No	HCP only
Simple CD2WB [B]	Whole blood	IgA TTG and AGA	Yes	No	HCP only
Celiac Quick Test[C]	Whole blood	IgA/IgG/IgM TTG	Yes	Yes	HCP only
Celiac Screen Professional[D]	Whole blood	IgA/IgG TTG	Yes	Yes	HCP only
Xeliac Test Pro[E]	Whole blood	IgA/IgG TTG	Yes	Yes	Public and HCP
Simtomax[F]	Whole blood, serum, and heparinised EDTA plasma	IgA/IgG DGP, total IgA	Yes	Yes	HCP only

A: Ani Biotech Oy, Finland; B: Operon S.A., Spain; C: Biohit Healthcare, UK; D: Personal Diagnostics, UK; E: Eurospital, Italy; F: Augurix Diagnostics, Switzerland

1.2.3: Current evidence for point of care tests for coeliac disease

The main body of work on point of care tests has been focussed on Biocard, which initially appeared to perform well with sensitivities over 90%. However, common flaws in the methods used seemed to have overestimated its efficacy. Firstly, most studies only biopsied patients with a positive serology or point of care test. For instance, Raivio's group examined the diagnostic accuracy of Biocard in 150 high risk patients (82). All patients had a Biocard test alongside EMA and TTG. Only those with positive serology (n=44) had duodenal biopsies which confirmed coeliac disease. The sensitivity and specificity of Biocard in concordance to serology were 95.5% and 97.1% respectively. The fact that only those with positive serology were biopsied, together with the high coeliac disease prevalence of 29.9%, would contribute to the apparently high sensitivities of Biocard, as false negatives would be missed. Additionally, the sensitivity and specificity of Biocard were compared against serology rather than duodenal histology as the reference standard, meaning that the true performance of Biocard was not reflected. Appendix 1 contains details of all the point of care studies to date, including their study limitations.

Secondly, a common issue is an inherent high pre-test probability due to tertiary referral bias. The coeliac disease prevalence of the case finding and case control studies ranged from 1.5-65.4% (median 38.5%), in contrast with 0.24-0.79% in population screening studies (see appendix 1). These limitations could overestimate the sensitivity of Biocard. As an example, Raivio's group conducted a subsequent similar but larger study evaluating Biocard using stored whole blood samples from 139 patients with coeliac disease and 103 controls (83). The sensitivity and specificity of Biocard were found to be 93% and 94%. However, we must take into account the

significantly high coeliac disease prevalence at 57.4%, which would cause a positive ascertainment bias through the high pre-test probability, thereby potentially falsely inflating the sensitivities of Biocard.

When Biocard was compared against duodenal histology as the reference standard in all patients, its sensitivities disappointingly fell to 70-80% (84, 85). Mooney's group evaluated the performance of Biocard in adults with suspected coeliac disease attending for a gastroscopy. All patients underwent EMA, TTG, Biocard and duodenal biopsies irrespective of serology or Biocard results. The prevalence of coeliac disease was moderately high at 22.4% due to a tertiary referral bias, but even so, the sensitivity dropped to 70.1% (84). The low sensitivity of Biocard was mirrored in another prospective case finding study in a paediatric population in India when the all-biopsy method was employed (85). They tested Biocard on 124 patients referred to the tertiary care centre with suspected coeliac disease, with a very high prevalence of coeliac disease at 64.7%. Nevertheless, the sensitivity and specificity still fell to 83% and 90% respectively when compared against histology.

It is noteworthy that the sensitivity of Biocard evaluated by a Finnish group (sensitivity 93%) (83, 86) was higher than the above mentioned studies (sensitivities 70-80%) (84, 85, 87), even though they all used serology and duodenal histology as the reference standard. This could partly be explained by the difference in the method of blood sample collection, where the Finnish group used stored whole blood samples from the laboratory instead of a fresh whole blood sample from a finger prick in the latter two groups. Because Biocard depends on the liberation of

endogenous TTG from red blood cells to act as the autoantigen for the test, insufficient red cells from a finger prick may liberate insufficient TTG to generate a positive result. Hence it is possible that the sensitivity of Biocard may be lower if a capillary finger prick sample is being used instead of a venous blood sample.

Other point of care tests detecting TTG such as Celiac Quick Test, Stick CD1/CD2 and Simple CD1WB/CD2WB appeared to be comparable to serology, but there are only a handful of studies assessing their diagnostic accuracy, with similar limitations as those of Biocard. Therefore, caution should be taken when interpreting these results. Although Simtomax likewise has not been extensively investigated, it has emerged to be the better performing point of care test, with sensitivities of 92.7-100% and specificities of 85.2-95% (87-90). However, only one study (87) biopsied all patients irrespective of their point of care test or serology results; two of the studies compared Simtomax against serology rather than duodenal histology (87-90); and all four studies had a high pre-test probability (9.6%-65.4%). Thus, the results may not be truly reflective of the performance of Simtomax in clinical practice. Nevertheless, Simtomax was shown to be the best point of care test in a head to head trial in the UK comparing Celiac Quick Test, Biocard, Simtomax and IgA TTG in 55 patients referred with a positive EMA (87). The prevalence was expectedly high at 65.4%, and the sensitivity was 77.8% for Celiac Quick Test, 72.2% for Biocard, 94.4% for Simtomax and 97.2% for TTG.

1.2.4: Gaps in the current literature

Simtomax has been shown to be a promising point of care test for coeliac disease. However, most study methods had limitations which could affect the true efficacy of Simtomax in clinical practice. Moreover, the prevalence of coeliac disease tended to be high in these studies due to tertiary referral bias. The sensitivities of Simtomax may drop when applied to a lower disease prevalence population. There is a lack of data in the current literature on the true diagnostic performance of Simtomax without positive ascertainment bias or tertiary referral bias.

1.2.5: Simtomax- a point of care test detecting IgA and IgG deamidated peptide antibodies

Simtomax stands out from the other point of care tests in which it detects IgA/IgG-DGP rather than IgA-TTG, as well as the presence of IgA. As previously mentioned, gliadin in gluten is deamidated by TTG to DGP, a process that enhances its binding to HLA DQ2 and HLA DQ8 receptors, which triggers a gut derived T cell immune response (31, 91, 92). DGP can be detected by Simtomax via different types of blood samples such as venous or finger prick capillary whole blood, serum and plasma. It is manufactured by Augurix in Swizterland, and is currently available for order by health care professionals in the UK through its UK distributor, Tillotts Pharma. There are four reports so far on Simtomax, all of them being case finding studies (87-90). Details regarding how Simtomax is used is described in the methods section in chapter two.

1.3: Summary

Coeliac disease is a common yet under diagnosed condition. One of the reasons may be due to deficiencies in case finding strategies with serological testing. If Simtomax can be shown to have diagnostic accuracy comparable to conventional serology, it may offer a more convenient and potentially more cost effective way of detecting and managing coeliac disease.

Chapter 2: Framework of thesis

2.1: Null hypothesis

The null hypothesis is that a point of care test has no role in the diagnosis and management of coeliac disease. In order to reject the null hypothesis, we will evaluate the utilisation of a point of care test, Simtomax, in four studies, which will form the chapters of my thesis.

2.2: Aims

Study 1: To assess the role of Simtomax as a primary care case finding tool for coeliac disease in high risk individuals in community pharmacies. (chapter 3)

Study 2: To demonstrate the diagnostic accuracy of Simtomax in secondary care, by recruiting patients with gastrointestinal symptoms or self-reported gluten sensitivity who were referred to secondary care. The sensitivities of Simtomax were compared to conventional serology with duodenal histology as the reference standard. Patient acceptability of Simtomax and inter-observer agreement of the Simtomax results were also evaluated. (chapter 4)

Study 3: To establish the diagnostic accuracy of Simtomax and its cost effectiveness of coeliac testing in patients with iron deficiency anaemia in the endoscopy setting. (chapter 5)

Study 4: To investigate whether Simtomax is a reliable surrogate marker for predicting histological remission in patients with known coeliac disease on a gluten free diet. (chapter 6)

2.3: General methodology

The methodology for all chapters involving Simtomax, coeliac serology and duodenal biopsies are described here to avoid repetition in subsequent chapters. Any additional methodological details will be provided in individual chapters.

2.3.1: The point of care test, Simtomax

The DGP based point of care test for celiac disease, Simtomax, was manufactured by Augurix Diagnostics, Rheinfelden, Switzerland. It detects both IgA/IgG-DGP antibodies, as well as the presence of IgA. The assay is based on lateral flow immunochromatography using colloidal gold antihuman antibodies as a signal detector. A sample of 25µl of capillary venous blood was obtained through a simple finger prick technique. The blood sample was then applied to the test device, followed by the application of five drops of the provided buffer solution. The result was available after ten minutes. Positive results were indicated by the presence of a solid red band for IgA/IgG-DGP positivity. A second single red band indicated the presence of IgA. A third in-built red control band ensured a correctly functioning test. See figure 5 for illustration of the point of care test.

Figure 5. How to perform a Simtomax test.

From left to right from the top down: The test kit comprises of an alcohol wipe, a lancet, a pipette, a test device, buffer solution and a plaster. The fingertip is pricked using the lancet, producing a droplet of blood. Capillary venous blood is drawn using the pipette until the blood reaches the black line on the pipette (25µl). The blood sample is expelled from the pipette and deposited into the outer window of the test device. After 30 seconds, the test device lid is lifted and five drops of the buffer solution is added to the circular port. Allow the test to run on a flat surface and wait 10 minutes before reading the results. The lid should not be closed while running the test. Positive results are indicated by the presence of a solid red band (A) for IgA/IgG-DGP positivity. A second single red band (B) indicates the presence of IgA. A third in-built red control band (C) ensures a correctly functioning test. The test result on the left is negative and the one on the right is positive.

The above photos were taken by myself.

2.3.2: Coeliac serology

IgA-TTG was previously assayed using ELISA kit (Aesku Diagnostics, Wendelsheim, Germany). Since 2014, IgA-TTG has been assayed using fluorescence enzyme immunoassay (Thermo Fisher Immunocap EliA, UK). A TTG titre of >7 U/ml was regarded as positive as per the manufacturer's guidance.

IgA-EMA was detected by immunofluorescence on primate oesophagus sections (Binding Site, Birmingham, UK). Total IgA was measured on a Behring BN2 nephelometer (Haywards Heath, West Sussex, UK).

2.3.3: Histological evaluation

In total, at least five biopsies were taken from the duodenum with a single bite per pass technique, including at least one biopsy from the duodenal bulb and four quadrantic biopsies from the second part of the duodenum. Each biopsy was fixed in formalin at the time of the gastroscopy. Specimens were then processed, orientated and embedded in paraffin wax by the pathology department. Standard 3µm thick sections at three levels were stained with haematoxylin and eosin, and reported by gastrointestinal histopathologists without knowledge of the point of care test or serology results. Villous atrophy was graded according to the modified Marsh criteria (93).

2.4: Statistical analysis

Data were summarised by descriptive statistics, including counts and percentages for categorical data, and medians and ranges for continuous parameters. The diagnostic accuracies of Simtomax, IgA-TTG and IgA-EMA were presented with sensitivity, specificity, PPV and NPV. Clopper-Pearson method was used to calculate the confidence intervals (CI) for the sensitivities.

Statistical analysis was performed using IBM SPSS statistics version 24.

2.5: Definitions of diagnoses

The definition of coeliac disease was based on positive serology (positive TTG and/or EMA) with Marsh 3 villous atrophy.

Seronegative coeliac disease was based on Marsh 3 villous atrophy on a normal gluten containing diet, positive HLA DQ2 or DQ8, and other supporting information such as family history and response to a gluten free diet. Non-coeliac causes of seronegative villous atrophy were extensively investigated for, including giardiasis, tuberculosis, Whipple's disease, small bowel bacterial overgrowth, helicobacter pylori, human immunodeficiency virus, autoimmune enteropathy and drug related causes.

Potential coeliac disease was defined as positive serology with no villous atrophy (Marsh 0-2),

with supporting information such as positive HLA DQ2 or DQ8 and family history.

Non-coeliac gluten sensitivity was diagnosed in patients who self-report symptoms related to gluten, with negative serology, absence of villous atrophy, and symptom induction by gluten challenge.

2.6: Reference standard

The reference standard for the diagnostic performance of coeliac serology and Simtomax: Marsh 3 villous atrophy secondary to coeliac disease (includes seronegative coeliac disease after other causes of seronegative villous atrophy were ruled out)

Chapter 3: The role of a point of care test in primary care: a case finding feasibility study

3.1: Abstract

Background: Coeliac disease affects 1% of the population, but 75% remain undiagnosed. This is partly due to coeliac serology being under-utilised, suggesting insufficiencies in current case finding strategies. The aim was to evaluate the case detection rate using a point of care test in primary care, and the uptake rate of Simtomax and subsequent gastroscopies.

Methods: A prospective study was performed in six community pharmacies across Sheffield, UK, using a point of care test, Simtomax (IgA/IgG-deamidated gliadin peptide). Pharmacy customers with symptoms suggestive of or risk factors for coeliac disease were tested with Simtomax. Positive individuals were referred for a gastroscopy with duodenal biopsies alongside conventional serology. Individuals with known coeliac disease, those on a gluten free diet or those who were investigated for coeliac disease were excluded.

Results: A total of 802 customers were approached. Eight customers (1.1%) already had a coeliac disease diagnosis and were therefore excluded. Five hundred customers who met the inclusion criteria participated in the study and were tested with Simtomax (369 females, 73.8%; age range 18-87, median age 49), with a positive rate of 7.2% (36/500). The Simtomax uptake rate was 63% (500/794). Twenty-seven positive participants (75%) underwent a gastroscopy with duodenal biopsies and serology. Two participants were diagnosed with coeliac disease, and one with potential coeliac disease, with a case detection rate of 0.6%.

Conclusion: There was good uptake for point of care testing, although the case detection rate was much lower than anticipated. Therefore, it has a limited role as a case finding tool in a community pharmacy setting in our cohort.

3.2: Introduction

One of the major challenges with coeliac disease is that a considerable proportion of patients are undiagnosed despite it being a prevalent condition. Sanders et al. reported that approximately one third of patients were seen by other medical or surgical specialties with coeliac related symptoms before being diagnosed, and the mean delay in diagnosis in the Sheffield cohort was 4.9 years (14). In the past two decades, there has been an increasing recognition of the changing presentation of coeliac disease, where patients can exhibit subtler signs, such as fatigue and anaemia. Thus there has been a drive to test for coeliac disease in at risk individuals in primary care.

Previous primary care case finding studies have shown improvement in case detection rates through screening high risk patients with serology. Sanders' group found 12 new cases of coeliac disease when screening 1200 patients with fatigue, anaemia and irritable bowel syndrome (94). Hin et al. screened 1000 high risk patients and newly diagnosed 30 patients with coeliac disease (prevalence 3%). They quoted a fourfold increase in new cases compared to seven new cases diagnosed in a local district general hospital in the previous year (95). Similarly, Catassi's group found a 2.3% case detection rate in 976 high risk patients, increasing the diagnostic rate from 0.27 to 11.6 per 1000 visits when compared to the preceding 12 months (16).

Nevertheless, despite the national guidelines to test for coeliac disease in patients with suggestive symptoms or risk factors, it appears that clinicians do not uniformly follow this practice. This was previously demonstrated in our multicentre study, where we collected data on the availability of

coeliac serology prior to gastroscopy with duodenal biopsies in 934 anaemic patients from four UK hospitals (Bradford, Whipps Cross, Addenbrooke's and Hull). We found that only 33.8% of patients had serology performed despite guideline recommendations (96). This result echoes the 31.8% availability rate of serology in patients with suspected coeliac disease undergoing a gastroscopy as demonstrated by Wiland's group in America (4).

Several reasons could explain the lower than expected usage of coeliac serology. The main reason is probably due to the fact that coeliac disease presents in an array of symptoms, some of which are vague and insidious. This makes it difficult for clinicians to recognise, and some patients accept these symptoms as part of their health. Clinicians also fail to recognise opportunities for testing, as illustrated by Sanders' group where patients had sought help from multiple health care professionals, and some had had gastroscopies without duodenal biopsies taken before being diagnosed with coeliac disease years later (14). Moreover, under the current climate of the NHS, increasingly longer waits to access primary care are not uncommon. Further delay is encountered with the arrangement of a blood test for coeliac serology, obtaining the results, and referral onwards for a gastroscopy. All these issues suggest insufficiencies in our current case finding strategy.

This necessitates an alternative approach to improve case detection. A point of care test offers the advantage of rapid result availability within 10 minutes, making it suitable for use in a community setting. A recent proof of concept study demonstrated that screening 551 high risk individuals with Simtomax in community pharmacies led to a positive Simtomax test in 9.4% of the participants.

However, there was no data pertaining to the subsequent follow up or biopsy results to confirm the number of coeliac disease cases detected (97).

3.3: Methods

Aims:

To evaluate the role of Simtomax for the detection of coeliac disease in at risk or symptomatic individuals in a primary care setting.

Study design and recruitment:

A prospective case finding study was conducted in six community pharmacies across the city of Sheffield (Darnall, Foxhill, Manor Top, Wicker, Barnsley Road) over a four-month period. Recruitment was achieved in three ways:

1. Customers at the pharmacies were approached by the principal investigator (PI) i.e. myself and I informed them about the study. Those who were interested were invited to a private consultation room within the pharmacies to determine eligibility of participation through completion of a participant's questionnaire (see appendix 2). Customers with symptoms or risk factors for coeliac disease indicated for coeliac screening by the NICE guidelines (see table 2) were recruited.

Table 2: Symptoms of and risk factors for coeliac disease that fulfilled the inclusion criteria for the study.

Persistent unexplained abdominal or gastrointestinal symptoms	Coeliac disease in first degree relatives
Irritable bowel syndrome	Metabolic bone disorders (osteomalacia/ osteoporosis)
Prolonged fatigue	Unexplained neurological symptoms (peripheral neuropathy or ataxia)
Unintentional weight loss	Unexplained subfertility or recurrent miscarriage
Severe or persistent mouth ulcers	Persistently raised liver enzymes with unknown cause
Iron, vitamin B12 or folate deficiency	Dental enamel defects
Type 1 diabetes	Autoimmune thyroid disease

2. Customers obtaining relevant medications either through prescription or over the counter were approached by the pharmacists and referred to the PI for study participation. The relevant medications included treatment for possible symptoms of coeliac disease (e.g. antispasmodics for irritable bowel) or conditions that may be associated with coeliac disease (e.g. insulin for type 1 diabetes). These target medications are listed in table 3.

3. Posters advertising for the study were in place in all pharmacies which customers could enquire further for study participation (see appendix 3).

Table 3: Types of medications taken by an individual that would trigger recruitment into the study.

Drug group	Example drugs	Association with coeliac disease
Antispasmodics	Mebeverine, buscopan, spasmonal	Irritable bowel syndrome
Antacids	Proton pump inhibitor, gaviscon, ranitidine	Dyspepsia
Anti-diarrhoeal	Loperamide	Diarrhoea
Laxatives	Senna, movicol, sodium docusate	Constipation
Anti-emetic	Domperidone, metoclopramide	Nausea and vomiting
Supplements	Ferrous sulphate, vitamin B12, folate	Iron, B12 and folate deficiencies
Thyroid medications	Thyroxine, carbimazole	Autoimmune thyroid disease
Insulin	All types of insulin	Type 1 diabetes

Inclusion criteria:

Individuals aged 18 or over, with any symptoms or risk factors listed in table 2, and/or taking medications listed in table 3.

Exclusion criteria:

Known coeliac disease, being on a gluten free diet, previous or current investigation for coeliac disease, and pregnancy.

Recruitment:

Individuals who met the eligibility criteria were approached and consented for the study. Participants were given background information about coeliac disease, explained the process of point of care testing, and the meaning of the test results.

Simtomax testing was performed in a private consultation room within the pharmacies. The results were obtained within 10 minutes and participants were informed of the results in real time. Those with a negative test were advised to see their general practitioner if necessary or if their symptoms persisted. Those with a positive test were referred for further investigations at the Royal Hallamshire Hospital, Sheffield.

Positive participants underwent conventional coeliac serology (IgA-TTG, IgA-EMA) and total IgA levels, followed by a gastroscopy with quadrantic duodenal biopsies from the second part of the duodenum and one duodenal bulb biopsy. A diagnosis of coeliac disease was made based on positive coeliac serology (EMA+/- TTG) plus villous atrophy on duodenal biopsies. Participants with coeliac disease ruled out were informed of their results and discharged to primary care. Those who were diagnosed with coeliac disease were offered a clinic appointment at the outpatient department at the Royal Hallamshire Hospital, and their management pathway was as per standard.

Outcome measures:

The primary outcome measure was the coeliac disease detection rate. Secondary outcomes included the uptake rate of Simtomax and subsequent investigations.

Sample size:

The sample size of this study was aimed at 500. The estimated prevalence of coeliac disease in this study cohort was 3%. This 3% prevalence was based on previous case finding studies in primary care: Hin et al demonstrated a 3% prevalence of coeliac disease in patients with high risk symptoms (95); and Sanders et al 2002 found a 3.3% prevalence of coeliac disease in participants with irritable bowel syndrome and fatigue in South Yorkshire (14).

Statistics:

The statistics department of the University of Sheffield was consulted regarding the sample size. Using an exact binomial test, a sample size of 500 would have 88% power to detect the difference between the estimated 3% coeliac disease prevalence in the study cohort and the estimated 1% prevalence of coeliac disease in the general population.

Ethics approval:

This study protocol was approved by the Yorkshire and the Humber Research Ethics committee and registered with the local research and development department of Sheffield Teaching

Hospital NHS Foundation Trust under the registration number STH19172. (see appendix 4 for patient information sheet and appendix 5 for consent form)

3.4: Results

Eight hundred and two pharmacy customers were approached for the study. Eight individuals (1.1%) who were approached already had a coeliac disease diagnosis and were therefore excluded. Five hundred individuals met the eligibility criteria and agreed to undertake the point of care test, giving a point of care test uptake rate of 63% (500/794). There were 369 females (73.8%), and the age range was 18-87 (median age 49). Table 4 illustrates the participants' presenting characteristics.

Table 4: Participants' presenting characteristics.

Presenting features	**Number of participants (n=500)**	**%**
Persistent unexplained abdominal or gastrointestinal symptoms	441	88.2
Irritable bowel syndrome	176	35.2
Prolonged fatigue	261	52.2
Unintentional weight loss	48	9.6
Severe or persistent mouth ulcers	68	13.6
Iron, vitamin B12 or folate deficiency	47	9.4
Type 1 diabetes	4	0.8
Autoimmune thyroid disease	47	9.4
Coeliac disease in first degree relatives	21	4.2
Metabolic bone disorders (osteomalacia/ osteoporosis)	20	4
Unexplained neurological symptoms (peripheral neuropathy or ataxia)	1	0.2
Unexplained subfertility or recurrent miscarriage	9	1.8
Persistently raised liver enzymes with unknown cause	2	0.4

Thirty-six participants (36/500=7.2%) were tested positive, of which 27 (75%) subsequently underwent further evaluation with conventional serology and a gastroscopy with duodenal biopsies. Of the remaining nine positive participants who did not have further investigations, seven participants changed their mind at the pharmacy and declined further investigations, one participant wanted time to consider further tests and eventually declined a gastroscopy, and one agreed to further tests but did not attend for their gastroscopy appointments and subsequently declined further tests over follow up phone calls.

Of the 27 participants with positive Simtomax who underwent further investigations, two participants were diagnosed with coeliac disease, and another with potential coeliac disease. One was a classical coeliac disease with positive EMA and TTG, and Marsh 3a histology. The other was a seronegative coeliac disease with negative EMA and TTG, normal IgA levels, Marsh 3b histology and positive HLA DQ2. Other causes of seronegative villous atrophy were excluded. This patient had known hypothyroidism, and presented with weight loss and persistent mouth ulcers, with a first degree relative with coeliac disease. The patient with potential coeliac disease had positive EMA and TTG and Marsh 0 histology. She was commenced on a gluten free diet in view of gluten related symptoms. The remaining 24 participants with positive Simtomax had negative EMA and TTG. The coeliac disease prevalence was 0.6% (3/500) including the patient with potential coeliac disease.

3.5: Discussion

This is the first study to demonstrate both the feasibility and efficacy of using a point of care test in community pharmacies for the detection of coeliac disease. Allied health care professionals are an asset for supporting and relieving the workload of clinicians. Existing models of healthcare have demonstrated that specialist nurses could provide effective care for patients with inflammatory bowel disease, for instance (98). The aim of offering a point of care test to at risk individuals at community pharmacies was to increase the detection of coeliac disease and reduce delays in diagnosis as an alternative approach in primary care.

In this study we have shown that there was a good response from pharmacy customers in regards to the uptake rate of the point of care test and subsequent further investigations as required. The positive point of care test rate was 7.2%, which was similar to the 9.4% positive rate from the previous proof of concept study using point of care testing in pharmacies by Urwin et al. (97), although no subsequent investigations were undertaken to confirm the diagnosis in that study.

The coeliac disease detection rate of 0.6% in our study was however much lower than anticipated. There are several possible reasons for this. One of the reasons could be because the existing caseload of coeliac disease is higher than average due to the presence of our tertiary referral centre for coeliac disease in Sheffield, and the heightened awareness for active coeliac testing by local general practitioners because of that. During the recruitment period, eight pharmacy customers approached for the study already had a known diagnosis of coeliac disease, giving a 1.1% known coeliac disease

prevalence among the 802 individuals approached, which is four times higher than the UK average detected prevalence of 0.24% (3). Nevertheless, a previous point of care test case finding study by Urwin et al. based in 15 pharmacies across England (97) had a similar Simtomax positive rate to our study, indicating that the low yield in primary care is probably not confined to our local area but beyond.

Another potential factor which may have contributed to the low detection rate is that 25% of participants who tested positive did not proceed to further investigations to confirm or exclude coeliac disease. Therefore, it is possible that undiagnosed cases have not been accounted for.

Lastly, most recruited individuals were passively approached to be offered point of care testing, as opposed to the individual actively seeking for medical advice for their symptoms. This may have led to the inclusion of individuals with mild or insignificant symptoms which they would not have sought for medical help in the first instance. It is conceivable that the case detection rate may possibly be higher if the point of care tests were performed in individuals with symptoms significant enough for them to actively request for coeliac testing at the pharmacies.

3.6: Conclusion

We have demonstrated the feasibility of using a point of care test for the detection of coeliac disease in the primary care sector in community pharmacies. Although there was good uptake for undertaking the point of care test among pharmacy customers, the point of care test did not

increase the case detection rate as anticipated. Therefore, it has a limited role as a case finding tool in a community pharmacy setting in our cohort.

Chapter 4: The role of a point of care test in secondary care: a diagnostic accuracy study

4.1: Abstract

Background: Patients with gastrointestinal symptoms are frequently referred for specialist consult in secondary care. Moreover, individuals who self-report gluten sensitivity is increasingly presenting themselves to health care professionals. Point of care testing in secondary care could potentially be useful in coeliac disease detection, and differentiating between coeliac disease from non-coeliac gluten sensitivity. The aim of this study was to assess and compare the diagnostic performance of Simtomax to conventional serology (IgA-TTG and IgA-EMA) for the detection of coeliac disease in symptomatic patients (group 1), and in patients who self-reported gluten sensitivity (group 2). Patient acceptability and inter-observer variability of the point of care test results were also evaluated.

Methods: From 2013-2017, patients referred to secondary care with gastrointestinal symptoms, anaemia and/or weight loss (group 1) and patients with self-reported gluten sensitivity with unknown coeliac disease status (group 2) were prospectively recruited. All patients had concurrent Simtomax, IgA-TTG, IgA-EMA, total IgA levels, and a gastroscopy with duodenal biopsies. Five hundred patients completed acceptability questionnaires, and inter-observer variability of the Simtomax results was compared among five clinical staff for 400 cases.

Results: Group 1: 1000 patients, 58.5% female, age range 16-91, median age 57. Forty-one patients (4.1%) were diagnosed with coeliac disease. The sensitivity of Simtomax, IgA-TTG and

IgA-EMA were 82.9%, 78.1% and 70.7%; the specificities were 85.4%, 96.3% and 99.8%. Group 2: 61 patients, 83% female; age range 17-73, median age 35. Simtomax had 100% sensitivity and negative predictive value (NPV) in detecting coeliac disease in group 2. Most patients preferred Simtomax to venepuncture (90.4% vs 2.8%). There was good inter-observer agreement on the Simtomax results with a Fleiss Kappa coefficient of 0.895.

Conclusion: Simtomax had comparable sensitivities to IgA-TTG, and correctly identified all coeliac disease cases in a gluten sensitive cohort. However, its low specificity may increase unnecessary investigations. Despite its advantage of convenience and rapid results, Simtomax may not add significant value to case finding for coeliac disease in secondary care.

4.2: Introduction

Coeliac disease has an increasingly global presence, with a prevalence of 0.3-2.4% of the general population worldwide (2, 24, 26, 99-103). It affects 1 in 100 in the United Kingdom, but only 24% are detected (3). Similar observations are also apparent in Europe (99), the United States (54) and beyond (104). This is partly because symptoms of coeliac disease can be non-specific and difficult for clinicians to recognise.

This is further compounded by an emerging clinical entity, non-coeliac gluten sensitivity (NCGS), which is clinically indistinguishable from coeliac disease (105, 106). NCGS describes individuals who complain of gastrointestinal (bloating, abdominal pain, diarrhoea) and/or non gastrointestinal symptoms (fatigue, headaches, foggy mind) related to gluten ingestion, where coeliac disease and wheat allergy have been excluded (107). Although the Salerno criteria define NCGS using a double blinded placebo controlled challenge (108), self-reported gluten sensitivity describes individuals who complain of gastrointestinal and/or non gastrointestinal symptoms triggered by gluten ingestion and present to physicians accordingly. There is a paucity of data on the prevalence of NCGS, but it has been reported that 13% of the general population in the UK self-report gluten sensitivity, although only 0.8% had a diagnosis of coeliac disease (109). The immunological pathway of NCGS is not yet fully elucidated. Currently. It is understood that NCGS is associated with an innate, rather than adaptive immune response triggered by gluten (110), unlike coeliac disease where both pathways are involved. A double blinded, placebo controlled dietary re-challenge trial showed evidence that patients with NCGS respond to a gluten free diet.

However, it was suggested that it may be the other components of wheat (fermentable, oligo-, di-, monosaccharides, and polyols) that contribute to the symptoms in patients with NCGS, rather than gluten itself (111). Exclusion of coeliac disease and wheat allergy is fundamental in this group of patients.

It is essential to distinguish NCGS from coeliac disease, as patients with NCGS do not seem to be at risk of the complications seen in coeliac disease, although they derive symptomatic benefit from a gluten free diet (112). Furthermore, any delays in coeliac testing before individuals embark on a self-imposed gluten free diet could cause diagnostic challenges.

Early diagnosis of coeliac disease is important for the improvement of patients' quality of life and the prevention of complications such as osteoporosis, hip fractures and lymphoproliferative malignancies. It has been shown that serological testing in patients with high risk symptoms in a clinic setting yielded 3.3-4.7% coeliac disease detection (94). Similar results were obtained by other groups through case finding (95, 113). On the other hand, a recent systematic review reported insufficient evidence to support screening for asymptomatic patients at present (105, 114-117).

For these reasons, case finding for coeliac disease in at risk individuals has been recommended by international guidelines (35, 36). However, serological testing for coeliac disease remains under-utilised (4, 118). This could be due to a multitude of factors, including a lack of awareness

of the guidelines, inconvenience and cost. A finger prick point of care test that provides convenience and rapid coeliac antibody results may have a role in improving case detection, particularly in secondary care consultations, where the results could potentially provide immediate guidance for the physician on the need for duodenal biopsies.

4.3: Methods

Aims:

To assess the diagnostic accuracy of Simtomax in the detection of coeliac disease in secondary care. The secondary aims were to evaluate patient acceptability of Simtomax, and the inter-observer variability of test result interpretation.

Study design and recruitment:

The study took place at the Royal Hallamshire Hospital, Sheffield, U.K., from March 2013-January 2017. Patients with gastrointestinal symptoms who were referred to gastroenterology for further evaluation were prospectively recruited. The coeliac disease status of these patients was unknown. All patients were concurrently tested with total IgA levels, IgA-TTG, IgA-EMA, and the point of care test, and duodenal biopsies were taken in all patients as the reference standard.

Inclusion criteria:

Group 1: patients presenting to secondary care with gastrointestinal symptoms (abdominal pain,

diarrhoea and/or dyspepsia), anaemia and/or weight loss.

Group 2: patients presenting to secondary care with self-reported gluten sensitivity, with gastrointestinal and/or extra-gastrointestinal symptoms related to gluten ingestion. Those with reduced or no gluten intake were asked to undertake a six-week gluten challenge of 10g gluten/day prior to their endoscopy as per guidelines (119, 120).

Exclusion criteria:

For groups 1 and 2, patients with known coeliac disease and patients on a self-imposed gluten free diet who declined a gluten challenge were excluded. Group 1 patients who were referred with positive coeliac serology by their primary care physicians were excluded from the study so as to avoid tertiary referral bias, thus providing a more accurate assessment of the sensitivities of the point of care test that is reflective of clinical practice.

Patient acceptability of the point of care test

There are no validated patient acceptability questionnaires in the literature for point of care tests. Therefore, a questionnaire consisting of five questions regarding the acceptability of the point of care test (comfort level, convenience and satisfaction with result availability) was devised. The questionnaires were filled in by 500 consecutive patients after having had Simtomax performed. They were asked to rate on a Likert scale of one to five for each question, with one being a negative experience and five being a positive experience. These 500 patients all had previous

experience of a venepuncture. They were also asked to state their preferred mode of testing: point of care test, venepuncture, or no preference. A similar acceptability questionnaire for venepuncture was completed by a separate cohort of 63 patients after having had a venepuncture to act as controls. These questionnaires were given out to both groups to fill in independently and anonymously, and the questionnaires were collected by a member of staff on completion. (See appendix 6 for the acceptability questionnaires)

Inter-observer variability of the point of care test results

Inter-observer variability of the point of care test results was assessed in 400 consecutive patients in group 1. Each observer recorded whether there was a definite red band, a faint red band, or an absence of a red band. There were five observers in total for each case, consisting of one gastroenterologist and four other randomly selected allied health care professionals (for example nurses). All observers were trained to recognise positive, negative and indeterminate results. Observation of the results was carried out indoors under fluorescent lighting.

Ethics approval:

The study protocol was approved by the Yorkshire and the Humber Research Ethics committee and registered with the local research and development department of Sheffield Teaching Hospital NHS Foundation Trust under the registration number STH15416. Written consent was

obtained from all patients. (See appendix 7 for patient information sheet and appendix 8 for consent form)

Statistical analysis:

Inter-observer variability was presented using Fleiss Kappa coefficient, where 0 indicates no agreement and 1 indicates perfect agreement.

Cohen's effect size (r) for patient acceptability between Simtomax and venepuncture groups was measured using Mann-Whitney U test, where r=0.1, 0.3 and 0.5 indicates small, medium and large effect size respectively.

Receiving operator characteristic (ROC) curves were constructed with paired sensitivities and specificities of each surrogate marker, and the area under the curve (AUC) was determined.

4.4: Results

Patient demographics and presenting characteristics in group 1 are illustrated in table 5. One thousand eligible patients who consented for participation entered group 1 of the study. There were 585/1000 females (58.5%); age range 16-91, median age 57.

Table 5: Group one patient demographics and presenting characteristics table.

	No. of patients	Coeliac disease yield
Female	585/1000 (58.5%)	27/585 (4.6%)
Male	415/1000 (41.5%)	14/415 (3.4%)
Diarrhoea	75/1000 (7.5%)	8/75 (10.7%)
Abdominal pain	159/1000 (15.9%)	13/159 (8.2%)
Weight loss	104/1000 (10.4%)	6/104 (5.8%)
Anaemia	194/1000 (19.4%)	9/194 (4.6%)
Dyspepsia	549/1000 (54.9%)	8/549 (1.5%)

Forty-one patients (4.1%) were diagnosed with coeliac disease. IgA deficiency detected by total IgA levels from the laboratory assay was found in 28 patients in groups one and two combined (28/1061=2.6%). Two IgA deficient patients were diagnosed with coeliac disease (2/60=3.3% of the total coeliac cohort i.e. groups 1+2), and both had a positive Simtomax test. Six patients (6/60=10%) had seronegative coeliac disease, with three patients testing positive for SImtomax and one of whom had IgA deficiency. The sensitivity of Simtomax was comparable to IgA-TTG and IgA-EMA (82.9% vs 78.1% vs 70.7% respectively). However, its specificity was significantly lower than IgA-TTG and IgA-EMA (85.9% vs 96.3% vs 99.8% respectively).

The diagnostic performance of Simtomax, IgA-TTG and IgA-EMA for group one are displayed in tables 6 and 7. ROC curves for the aforementioned tests for group one are demonstrated in figure 6.

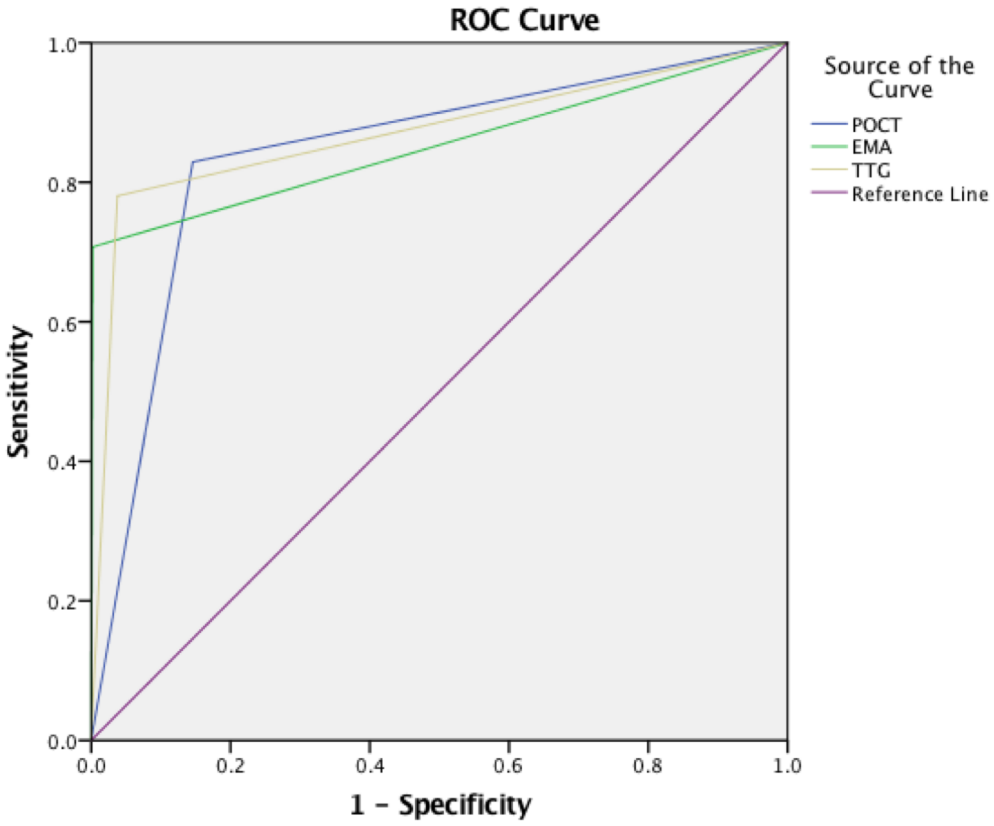
Table 6: The diagnostic accuracy of Simtomax, IgA-TTG and IgA-EMA in detecting coeliac disease in symptomatic patients (group one; n=1000, coeliac disease prevalence 4.1%).

	Simtomax	**IgA-TTG**	**IgA-EMA**
Sensitivity % (95% CI)	82.9 (67.9-92.9)	78.1 (62.4-89.4)	70.7 (54.5-83.9)
Specificity % (95% CI)	85.4 (83.0-87.6)	96.3 (94.8-97.4)	99.8 (99.3-100.0)
PPV % (95% CI)	19.5 (16.5-23.0)	47.1 (38.3-56.0)	93.6 (78.2-98.3)
NPV % (95% CI)	99.2 (98.4-99.6)	99.0 (98.3-99.5)	98.8 (98.0-99.2)
Positive likelihood ratio (95% CI)	5.7 (4.6-7.0)	20.8 (14.5-30.0)	339.2 (83.8-1373.2)
Negative likelihood ratio (95% CI)	0.2 (0.1-0.4)	0.2 (0.1-0.4)	0.3 (0.2-0.5)
Accuracy % (95% CI)	85.3 (83.0-87.4)	95.5 (94.0-96.7)	98.6 (97.7-99.2)

Table 7. Group 1: cross tabulation of Simtomax results by the reference standard.

	CD	**Not CD**		**CD**	**Not CD**		**CD**	**Not CD**
Simtomax +	34	140	**TTG +**	32	36	**EMA +**	29	2
Simtomax -	7	819	**TTG -**	9	923	**EMA -**	12	957

Figure 6. Group 1 ROC curve for Simtomax, IgA-EMA and IgA-TTG in symptomatic patients. Area under the curve (AUC) for each test were 0.842 (CI: 0.77-0.9), 0.853 (CI: 0.77-0.94) and 0.871 (CI: 0.8-0.95).



In group 2, 70 patients who self-reported gluten sensitivity were recruited. Nine patients who were on a self-imposed gluten free diet and declined a six-week gluten challenge prior to investigations were excluded from the study. A total of 61 patients consuming gluten entered group two of our study. There were 51/61 females (82.9%); age range 17-73, median age 35.

Twenty-three patients who were previously on a self-imposed gluten free diet underwent a gluten challenge: 16 patients managed a six-week challenge and seven could only tolerate four weeks of gluten challenge at which point the serology and endoscopy were performed due to

significant symptoms. The remaining 38 patients were consuming a gluten containing diet and continued to do so at least until the investigations took place. Eighteen patients were tested positive for EMA by their general practitioners. The vast majority (57/61=93.4%) of patients had gastrointestinal symptoms, and 10 patients (10/61=16.4%) reported extra-gastrointestinal symptoms, predominantly neurological complaints (e.g. headache, paraesthesia, foggy mind, ataxia, lethargy, tongue tingling and arthralgia).

Forty-two patients (42/61=68.9%) were diagnosed with NCGS, 17 (17/61=27.9%) with coeliac disease and two (2/61=3.3%) with potential coeliac disease. The point of care test demonstrated a sensitivity and NPV of 100% (vs sensitivities of 88.2%, 94.1% and NPV of 91.8%, 97.77% for IgA-TTG and IgA-EMA respectively). The diagnostic performance of Simtomax, IgA-TTG and IgA-EMA for group two are displayed in tables 8 and 9. ROC curves for the aforementioned tests for group two are demonstrated in figure 7.

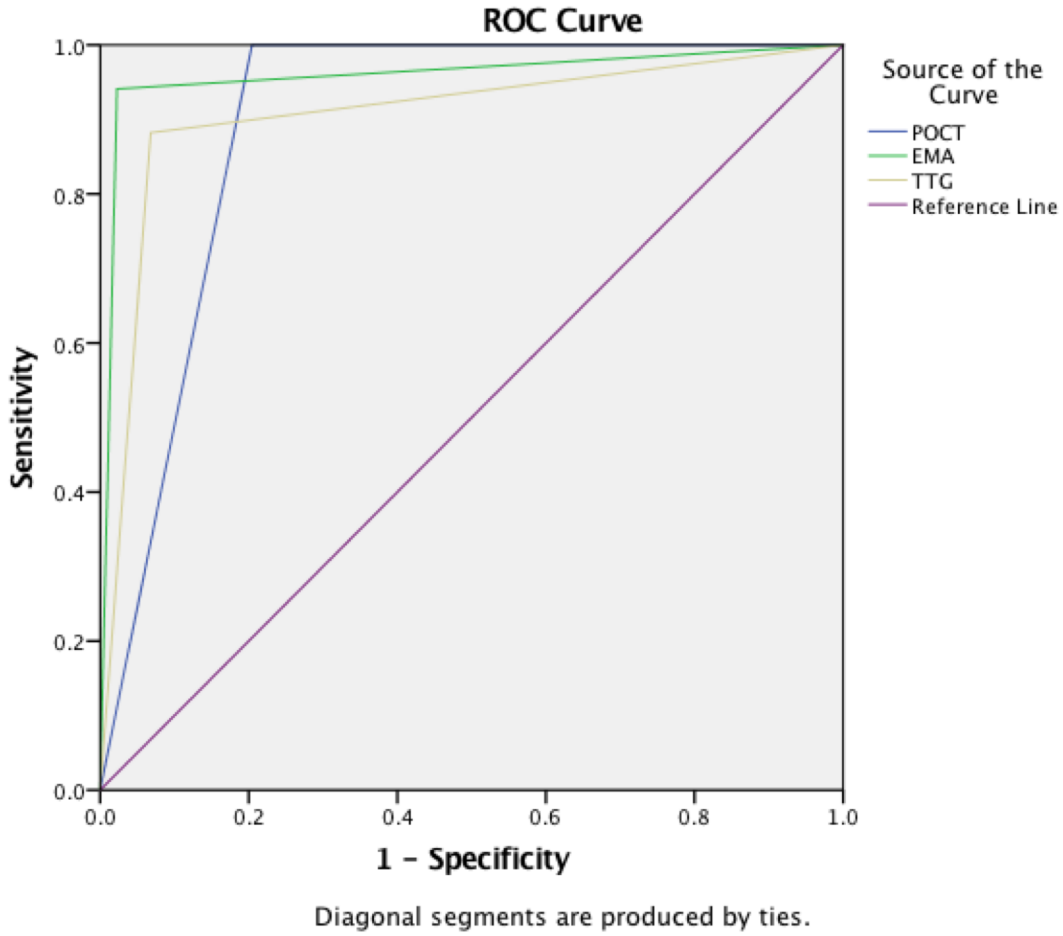
Table 8: The diagnostic accuracy of Simtomax, IgA-TTG and IgA-EMA in detecting coeliac disease in patients who self-reported gluten sensitivity (group two; n=61, coeliac disease prevalence 27.9%).

	Simtomax	**IgA-TTG**	**IgA-EMA**
Sensitivity % (95% CI)	100 (80.5-100)	88.2 (63.6-98.5)	94.1 (71.3-99.9)
Specificity % (95% CI)	79.6 (64.7-90.2)	93.2 (81.3-98.6)	97.7 (88.0-99.9)
PPV % (95% CI)	65.4 (51.3-77.2)	83.3 (62.3-93.8)	94.1 (69.6-99.1)
NPV % (95% CI)	100	91.8 (81.0-97.3)	97.7 (86.5-99.6)
Positive likelihood ratio (95% CI)	4.9 (2.7-8.8)	12.9 (4.3-39.1)	41.4 (5.9-288.5)
Negative likelihood ratio (95% CI)	0	0.1 (0-0.5)	0.06 (0.01-0.4)
Accuracy % (95% CI)	85.3 (73.8-93.0)	91.8 (81.9-97.3)	96.7 (88.7-99.6)

Table 9. Group 2: cross tabulation of Simtomax results by the reference standard.

	CD	Not CD		CD	Not CD		CD	Not CD
Simtomax +	17	9	**TTG +**	15	3	**EMA +**	16	1
Simtomax -	0	35	**TTG -**	2	41	**EMA -**	1	43

Figure 7. Group 2 ROC curve for Simtomax, IgA-EMA and IgA-TTG in patients who self-reported gluten sensitivity. AUC for each test were 0.898 (CI: 0.82-0.98), 0.959 (CI: 0.89-1.0) and 0.907 (CI: 0.81-1.0).



In regards to patient acceptability, Simtomax had significantly higher patient satisfaction compared to venepuncture. The difference in the scores between the two groups were statistically significant in all aspects of the acceptability questionnaire, and the effect size difference between the two groups was large (r = 0.506-0.656). Table 10 shows the median scores and statistical differences in both groups for each aspect of the tests.

Table 10: Patient acceptability for Simtomax and conventional venepuncture. Acceptability was scored with a Likert scale from 1-5, with 1 being a negative experience and 5 being a positive experience.

	Simtomax	**Venepuncture**	**Mann-Whitney U test**
Blood collection process:			
Score for comfort level of the test	4.7	3.3	U=2988.5, Z=12.027, p<0.001, r=0.506
Score for speed and ease of the test	4.7	3.3	U=2182.5, Z=13.443, p<0.001, r=0.566
Convenience:			
Satisfaction score for having the test performed during the consultation (for Simtomax) vs separately from the consultation by the phlebotomy service (for venepuncture)	4.8	2.9	U=1086.5, Z=14.675, p<0.001, r=0.617
Quality of care:			
Satisfaction score for obtaining test results within 10 minutes (for Simtomax) vs a few days to a week (for venepuncture)	4.8	3.1	U=583.5, Z=15.597, p<0.001, r=0.656
Satisfaction score for obtaining and discussing the test results with the clinician within the same consultation (for Simtomax) vs at a later date (for venepuncture)	4.8	2.9	U= 988.0, Z=15.223, p<0.001, r=0.64

Preference:	No. of patients
Prefers Simtomax	452/500 (90.4%)
Prefers venepuncture	14/500 (2.8%)
No preference	34/500 (6.8%)

There was a good degree of inter-observer agreement on Simtomax result interpretation, with a Fleiss Kappa coefficient of 0.895 overall. Sub-analysis revealed a high level of agreement for definite red bands (Kappa 0.887) and absence of red bands (Kappa 0.956). The level of agreement dropped for faint red bands (Kappa 0.781), where there were 31 such cases within the 400 assessed. None of these 31 patients had coeliac disease. Only solid red bands were classified as a positive test for the purpose of diagnostic calculations in the thesis, and faint red bands were interpreted as negative. Figure 8 illustrates the three possible outcomes of the point of care tests results.

Figure 8: Three possible outcomes of Simtomax results.

Red band A indicates a positive result, red band B indicates the presence of IgA, red band CT is the control line, indicating a correctly functioning test. Left: A solid red band A indicating a positive test; Middle: An absence of a red band A indicating a negative test; Right: A faint pink band A which was classified as a negative test, as none of the patients with a faint band A had coeliac disease in our cohort.

The above photographs were taken by myself.

4.5: Discussion

To my knowledge, this is the largest study to date evaluating the diagnostic accuracy of Simtomax. This is also the first study to explore the practicalities of this point of care test including patient acceptability and inter-observer variability of test result interpretation.

One of the strengths of this study is that all participants had duodenal biopsies taken, irrespective of their coeliac antibodies or Simtomax results. This ensured that no false negative cases of coeliac disease would be missed. This methodology contributed to a major difference to most point of care test studies for coeliac disease, where only patients with positive antibodies (either point of care test or serology) were biopsied (82, 88-90, 121, 122). Additionally, some point of care test studies measured the sensitivities against serology rather than duodenal histology as the reference standard (82, 123, 124). These methodological weaknesses could lead to a positive ascertainment bias, thereby falsely elevating the reported sensitivities.

Another strength of this study is that the patient cohort had a coeliac disease prevalence consistent with real life case finding in patients with high risk symptoms, which have been reported to be 3-4.7% (94, 95). A much higher coeliac disease prevalence is a common flaw in previous point of care test studies (85, 88, 125). This tertiary referral bias restricts the generalisability of their findings. The patient characteristics and pre-test probability of group 1 allowed a more accurate reflection of the diagnostic performance of these tests when used in clinical practice.

There are a few limitations in this study. Ideally, the measurement of laboratory DGP serology would act as a useful comparison of the sensitivities between DGP detection by laboratory assay and lateral flow immunochromatography. However, laboratory DGP serology is not widely available in the UK and is not available in our centre. Therefore, DGP assays were not performed.

Another limitation is the evaluation of patient acceptability of Simtomax. I devised the point of care test acceptability questionnaire as there were no validated questionnaires for point of care tests in the literature. Furthermore, the methodology of using a Likert scale provided a quantitative rather than qualitative measure of acceptability. Qualitative interviews would provide a more informative representation of patient acceptability. However, patient acceptability was a secondary outcome and not the main focus of this study.

What is noteworthy is the generally lower sensitivities of IgA-TTG and IgA-EMA compared to previous serological studies (126, 127). There are several potential reasons for this. Although a systematic review in 2006 showed that the pooled sensitivities of IgA-TTG and IgA-EMA from published data were 93% (range 70-100%) and specificities were >98% (range 90-100%) for both, the authors indicated that these figures were likely to be falsely high due to methodological shortcomings in most studies (126). Firstly, many studies did not biopsy controls (i.e. take duodenal biopsies in seronegative patients), potentially missing false negative cases. In a subsequent meta-analysis of the diagnostic accuracy of IgA-TTG and IgA-DGP (127), the authors concluded that only two out of 11 studies biopsied controls (128, 129). In fact, these two studies

demonstrated the sensitivities and specificities of IgA-TTG at 78.3-95% and 97.5-98.4% respectively.

Second of all, the results from the aforementioned two studies still may not have reflected their performance in real practice, as has been demonstrated previously (130), since the coeliac disease prevalence was very high at 74% for both studies. This again could have falsely increased the sensitivity and PPV of IgA-TTG due to positive ascertainment bias.

Lastly, the lack of standardisation of IgA-TTG laboratory assays could also lead to different IgA-TTG sensitivities. IgA-TTG antibody units and reference ranges are arbitrary and method-specific. Furthermore, over 30 different IgA-TTG assay kits are used in the UK, giving different IgA-TTG titres. A recent study showed that even when the same IgA-TTG ELISA assay kit was used, there was still poor agreement among laboratories as to whether the sample was above or below the defined IgA-TTG level cut off point for Marsh 3 histology using a ROC curve (131). A recent head to head trial of three different TTG serological kits also found widely variable sensitivities and specificities, ranging from 71.1%-95.5% and 82.6%-100% respectively (132).

All these factors explain the huge variability of IgA-TTG sensitivities and why the sensitivities appeared to be lower than average in this study, where all patients were biopsied including controls, and the coeliac disease prevalence being much lower in comparison to previous studies. In regards to the prevalence of coeliac disease in individuals who self-report gluten sensitivity, there are four studies in the literature which assessed the diagnostic outcomes of this cohort.

The sample sizes ranged from 93 to 238, and the prevalence of coeliac disease varied between 2% and 42.4% (109, 133-135). In our study, the coeliac disease prevalence of 27.9% in the self-reported gluten sensitivity cohort lies within the range of the reported data. The wide variation in the reported disease prevalence is likely due to differences in the study population, study design, recruitment methods and diagnostic criteria. For example, our disease prevalence of 27.9% is higher than the 7% reported by Aziz et al. which was derived from a UK population-based questionnaire targeting individuals with gluten related symptoms (109), as opposed to symptomatic individuals actively presenting to primary care who were then referred on to secondary care for further evaluation. Our group 2 patients' gluten related symptoms may have prompted more proactive coeliac screening by their general practitioners, thus possibly explaining the higher prevalence of seropositive patients (18/61), giving a higher disease prevalence. Nevertheless, after excluding the 18 patients who were referred with positive EMA, the sensitivity and NPV of Simtomax remained at 100%, where all four cases of coeliac disease were correctly identified.

Point of care tests for other laboratory measurements such as human immunodeficiency virus and international normalised ratio have been widely adopted in national practice in both primary and secondary care settings, owing to their clinical effectiveness and good patient acceptability (136, 137). Simtomax has been demonstrated to have favourable acceptability to patients compared to venepuncture, with 90.8% patients preferring Simtomax to venepuncture. Most patients generally found Simtomax to be a simple and quick test to perform (it took on average

one minute to perform the test, and 10 minutes for the results to become available), and less painful than venepuncture.

With regards to the diagnostic performance of Simtomax, the sensitivity was comparable to IgA-TTG (82.9% vs 78.1%). In the group 1 cohort, 7.3% (3/41) of the newly diagnosed patients had seronegative coeliac disease detected by Simtomax alone whilst IgA-TTG was negative. An increase in diagnostic yield was also demonstrated by Hoerter et al. recently, where the use of IgA-DGP serology resulted in a 15% increase in coeliac disease detection where IgA-TTG was negative (138). However, the specificity and PPV of Simtomax were inferior to IgA-TTG (85.4% vs 96.3% and 19.5% vs 47.1% respectively), due to a higher rate of false positives. This could potentially lead to unnecessary further investigations. A possible explanation of the low specificity is that approximately half of the group 1 cohort had dyspepsia, which constituted low risk for coeliac disease, and hence may have lowered the pre-test probability of coeliac disease and hence the PPV. On the other hand, when Simtomax was used in higher risk groups, such as patients who self-reported gluten sensitivity (group 2), the PPV increased to 65.4%, with a 100% sensitivity and NPV in detecting coeliac disease.

The advantages of Simtomax over conventional serology are favourable patient acceptability and rapidly available results in real time. Nevertheless, despite there being no significant difference in the overall diagnostic performance between Simtomax and serology based on test accuracy and ROC curve analysis, one must consider the clinical impact of the high false positive rates of Simtomax. The potential burden of a considerable increase in unnecessary investigations may

outweigh the benefits of a sensitive and convenient test. As a case finding tool in the secondary care setting where the coeliac disease prevalence would be expected to be approximately 4%, as was in this study and other case finding studies based on symptomatic cohorts (94), Simtomax may not provide significant added value compared to conventional serology due to its low specificity, albeit its similar sensitivity to IgA-TTG.

4.6: Conclusion

Simtomax had comparable sensitivities to IgA-TTG in detecting coeliac disease in symptomatic patients, and correctly identified all cases of coeliac disease in a gluten sensitive cohort that was consuming gluten. It also has the advantage of convenience, rapid result availability, and good patient acceptability. However, Simtomax is limited by its low specificity which may increase the number of unnecessary investigations. Simtomax therefore may not add significant value when used for case finding in secondary care in patients with gastrointestinal symptoms or risk factors compared to conventional serology.

Chapter 5: The role of a point of care test in iron deficiency anaemia at endoscopy: a diagnostic accuracy and a cost saving model

5.1: Abstract

Background

International guidelines recommend coeliac serology in iron deficiency anaemia, and duodenal biopsy for those tested positive to diagnose coeliac disease. However, pre-endoscopy serology is often unavailable, thus committing endoscopists to take routine duodenal biopsies. Some endoscopists consider duodenal biopsy mandatory in anaemia to exclude other pathologies. It is hypothesised that using a point of care test at endoscopy could fill this gap, by providing rapid results to target which anaemic patients require biopsies, and save costs by biopsy avoidance. Three key aspects to this hypothesis were thus evaluated: 1) to establish the availability of pre-endoscopy serology in anaemia; 2) to determine the sensitivities and cost effectiveness of pre-endoscopy coeliac screening with Simtomax in anaemia; 3) to explore whether other anaemia-related pathologies could be missed by this targeted-biopsy approach.

Methods

Group 1: pre-endoscopy serology availability was retrospectively analysed in a multicentre cohort of 934 anaemic patients at four UK hospitals. Group 2: the sensitivities of Simtomax, IgA-TTG and IgA-EMA were compared in 133 prospectively recruited patients with iron deficiency anaemia attending for a gastroscopy. The sensitivities were measured against duodenal histology as the reference standard in all patients. The cost effectiveness of Simtomax was calculated based on the number of biopsies that could have been avoided compared to an all-biopsy approach. Group 3: the duodenal histology of 153 patients presenting to a separate iron deficiency anaemia clinic were retrospectively reviewed.

Results

In group 1, coeliac serology was available in 361 (33.8%) patients. In group 2, the sensitivity and NPV were 100% and 100% for Simtomax, 96.2% and 98.9% for IgA-TTG, and 84.6% and 96.4% for IgA-EMA respectively. In group 3, the duodenal histology found no causes for anaemia other than coeliac disease.

Conclusion

Simtomax had an excellent diagnostic accuracy in iron deficiency anaemia and was comparable to conventional serology. Duodenal biopsy did not identify any causes other than coeliac disease for iron deficiency anaemia, suggesting that biopsy avoidance in Simtomax negative anaemic patients is unlikely to miss other anaemia-related pathologies. Due to its 100% NPV, Simtomax could reduce unnecessary biopsies by 66% if only those with a positive Simtomax were biopsied, potentially saving £3690/100 gastroscopies.

5.2: Introduction

One of the common presenting symptoms of coeliac disease is anaemia, affecting 15-26.8% of untreated patients (139, 140). It is usually caused by malabsorption, leading to iron, folate, and B12 deficiency (141). One way to increase the detection of coeliac disease is by screening individuals with iron deficiency anaemia, which affects 2-5% of the general population in the developed world (142, 143). At the endoscopy setting, 2.6-8.7% of patients presenting with anaemia are diagnosed with coeliac disease, although the data is sparse and mainly from small cohorts (140, 144-148). The current BSG iron deficiency anaemia guidelines recommend routine screening for coeliac disease with coeliac serology, and a gastroscopy with duodenal biopsy to confirm the diagnosis of coeliac disease if serology is positive (149).

Anecdotally, the availability and utilisation of coeliac serology prior to endoscopy appears to be highly variable, thus committing clinicians to take duodenal biopsies if serology results are unavailable in order to avoid missing the diagnosis. However, this is an expensive way of case detection. A recent Swedish study showed that routine duodenal biopsy was ineffective, with a number needed to biopsy of 577 to detect one case of coeliac disease, spending more than €30,000 per case (140). In an attempt to target patients who require a duodenal biopsy, Hopper et al. devised a clinical decision tool using a combination of pre-endoscopy serological testing and symptom assessment (150). This validated algorithm resulted in a 100% sensitivity and NPV in detecting coeliac disease when applied to 2000 prospectively recruited patients. Yet, the lack of serology availability prior to endoscopy in real life seemed to have precluded the widespread

utilisation of this effective and cost saving clinical decision tool. One method of filling the gap of unavailable serology is by using a point of care test at endoscopy.

5.3: Methods

Aims:

The aim of this study was to evaluate the role of performing a pre-endoscopy point of care test in iron deficiency anaemia in the endoscopy setting as a cost saving model.

Firstly, the rates of adherence to the BSG guidelines on coeliac screening in iron deficiency anaemia in clinical practice was reviewed, to demonstrate the pre-endoscopy availability of serology.

Secondly, the diagnostic performance of Simtomax in detecting coeliac disease in iron deficiency was ascertained, and the economic impact of using Simtomax as a screening tool to target biopsy taking only in those tested positive for Simtomax was evaluated.

Finally, a retrospective analysis was performed to explore whether routine duodenal biopsy would yield any alternative causes for iron deficiency anaemia other than coeliac disease, in order to assess whether targeting biopsies only in Simtomax positive anaemic patients would miss other duodenal pathologies causing anaemia. Figure 9 illustrates a flow diagram of how a point of care test in endoscopy would fit in.

Figure 9: A flow diagram of how a point of care test in endoscopy would fit in within the endoscopy setting.

Starting from the top second left picture in a clockwise fashion: The patient enters the endoscopy unit, undertakes a Simtomax test performed by a healthcare professional if iron deficiency anaemia is present. If the patient has a positive Simtomax, the endoscopist would take duodenal biopsies to exclude coeliac disease. If the patient has a negative Simtomax, duodenal biopsies would not be required.

The above photographs were taken by Professor Sanders.

Study design and recruitment:

Group 1 was a multicentre retrospective analysis of all patients with anaemia attending for a gastroscopy with duodenal biopsy at four UK hospitals (Addenbrooke's, Bradford, Hull and

Whipps Cross) over a 12 month period ranging from 2012 to 2014. The availability of coeliac serology prior to gastroscopy was reviewed.

Group 2 was a prospective study comparing the sensitivities of Simtomax to conventional serology in an iron deficient cohort. Patients with iron deficiency with or without anaemia were prospectively recruited. These patients attended for a gastroscopy at the Royal Hallamshire Hospital between 2013 and 2015. All recruited patients were consented for the study, and undertook Simtomax, IgA-TTG, IgA-EMA, total IgA levels, and a gastroscopy with quadrantic duodenal biopsy from the second part of the duodenum and at least one duodenal bulb biopsy.

Group 3 was a retrospective analysis of patients attending a separate non-coeliac specialised iron deficiency anaemia clinic at the Northern General Hospital in 2013-2014. Their duodenal histology and hospital case notes were reviewed to determine the yield of alternative causes other than coeliac disease in the context of iron deficiency anaemia. The decision to review the duodenal histology of patients attending a non-coeliac specialised iron deficiency anaemia clinic at the Northern General Hospital was so that the results would represent real world data without tertiary referral bias.

Inclusion criteria for group 2:

Patients with iron deficiency with or without anaemia.

Exclusion criteria for group 2:

Patients with known coeliac disease, those on a gluten free diet, patients with coagulopathy, active gastrointestinal bleeding or a suspected carcinoma observed during the examination were excluded.

Outcome measures:

The primary outcome was to assess the diagnostic accuracy of Simtomax in detecting coeliac disease in patients with anaemia.

Secondary outcomes included an economic analysis of the cost savings achieved by only performing duodenal biopsies in anaemic patients who had a positive Simtomax compared to routine biopsies for all anaemic patients attending for a gastroscopy; and a retrospective analysis of the duodenal histological findings in a separate cohort of patients with iron deficiency anaemia.

Economic analysis:

The financial impact at a local and national level of using Simtomax to avoid routine duodenal biopsies in anaemia was evaluated. In the UK, the Health Resource Group (HRG) tariff (payment from the NHS commissioners to hospitals for providing a service) for a gastroscopy with duodenal biopsy and a gastroscopy alone are £382 and £344 respectively. This means that £38 would be saved for the NHS budget if duodenal biopsy was avoided for each gastroscopy performed. On the other hand, there is cross charging between departments for each service provided. At the Royal Hallamshire Hospital, the histopathology department charges the gastroenterology

department £86 for the service of analysing four D2 biopsies and one D1 biopsy (local tariff may vary among different trusts).

Statistics:

As the PPV and NPV of a test are influenced by the pre-test probability, positive (PLR) and negative likelihood ratios (NLR) were also calculated as these are less prone to influence by disease prevalence.

Ethics approval:

The group 2 study protocol was approved by the Yorkshire and the Humber Research Ethics committee and registered with the local research and development department of Sheffield Teaching Hospital NHS Foundation Trust under the registration number STH15416. (See appendix 7 for patient information sheet and appendix 8 for consent form)

5.4: Results

In group 1, a total of 934 patients with anaemia underwent a gastroscopy with duodenal biopsy at four UK hospitals. Coeliac serology was only available in 315 patients (33.8%) prior to endoscopy. Forty-four (14%) serology samples were performed in primary care.

In group 2, 133 patients (88 females, 66%) with iron deficiency attending for a gastroscopy at the Royal Hallamshire Hospital were prospectively recruited (age range: 18-89 years, median 53). Twenty-six patients (19.5%) were diagnosed with coeliac disease. Simtomax correctly identified all cases of coeliac disease, defined by a combination of positive serology (IgA-EMA/IgA-TTG) and Marsh 3 villous atrophy. The results are shown in tables 11 and 12. There was one case of a 38 year old Zambian lady with seronegative villous atrophy secondary to tuberculosis. A comparison of the sensitivities of Simtomax, IgA-TTG and IgA-EMA are shown in table 13.

Table 11: Group 2 iron deficient patient characteristics and corresponding histology results.

	Simtomax positive	EMA positive	TTG positive	M*0	M1	M2	M3a	M3b	M3c	Coeliac disease
Iron deficiency anaemia (n=81)	30	13	19	55	7	1	2**	5	10	16 (19.8%)
Iron deficiency without anaemia (n=52)	15	10	15	33	8	1	2	2	6	10 (19.2%)

*Marsh grade
**One patient had seronegative Marsh grade 3a villous atrophy which was secondary to tuberculosis instead of coeliac disease.

Table 12: Cross tabulation of Simtomax results by serology and duodenal histology results in iron deficiency.

	Simtomax positive	Simtomax negative
Coeliac disease	26	0
No coeliac disease	19	88

Table 13: The sensitivities of Simtomax, IgA-TTG and IgA-EMA in group 2 in iron deficiency.

	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Simtomax	100 (86.8-100.0)	82.2 (73.7-89.0)	57.8 (42.2-72.3)	100 (95.9-100.0)
IgA-TTG	96.2 (80.4-99.9)	91.5 (84.5-96.4)	73.5 (55.6-87.1)	99.0 (94.5-100.0)
IgA-EMA	84.6 (65.1-95.6)	99.1 (94.9-100.0)	95.7 (78.1-99.9)	96.4 (91.0-99.0)

In group 3, 215 patients with iron deficiency anaemia attended a separate non-coeliac specialised anaemia clinic at the Northern General Hospital for investigation from 2013-2014. A total of 175 patients underwent a gastroscopy, and 153 of those had a duodenal biopsy. The duodenal histology samples of these 153 patients were analysed. Two patients had Marsh grade 3 villous atrophy on histology- one had a positive coeliac serology and hence was diagnosed with coeliac disease; the other patient was found to have a colonic tumour during the course of the investigation and subsequently died. He never had coeliac serology or HLA genotyping to confirm the diagnosis. Assuming the latter case to be coeliac disease, the prevalence of coeliac disease in group 3 would be 1.3%.

In group 3, 92.2% patients (141/153) had normal duodenal histology. Seven patients (4.6%) had lymphocytic duodenosis (Marsh grade 1) on their histology, all of whom had negative coeliac serology. Their hospital case notes were reviewed and screened for drug causes for lymphocytic duodenosis such as aspirin, proton pump inhibitors, olmesartan, non-steroidal anti-inflammatories and chemotherapy; autoimmune associations such as type 1 diabetes and thyroid disorders; and infections such as Helicobacter pylori, Whipple's, Giardia etc. Six of these patients had a cause for or association with lymphocytic duodenosis: vitiligo, autoimmune hypothyroidism, multiple sclerosis, aspirin use, proton pump inhibitor use and Helicobacter pylori infection respectively. No attributable cause was found for lymphocytic duodenosis in the remaining one patient, whose helicobacter status was unknown. The remaining three patients had reactive changes, chronic duodenitis and submucosal haemangioma respectively on their duodenal histology which were not the cause for their iron deficiency anaemia. Table 14 shows a breakdown of the significant findings on gastroscopy in the group 3 cohort. This table excludes hiatus hernia, mild oesophagitis (grade A), mild or non Helicobacter pylori (HP) related gastritis or duodenitis, hyperplastic polyps, cystic fundal polyps and lipoma, as these are incidental findings that did not alter clinical management. Significant findings that contributed to anaemia formed 9.2% (14/153) of the cohort, whilst significant findings that were unrelated to anaemia constituted 7.8% (12/153) of the cohort. Interestingly, no malignancy was found on gastroscopy in this cohort. The total yield of significant findings on gastroscopy was 16.3% (25/153).

Table 14. Significant findings on gastroscopy in the group 3 cohort.

Gastroscopy findings	No. of patients (%)	Cause of anaemia
HP related gastritis	9 (5.9%)	Yes
Peptic ulcers (both positive for HP)	2 (1.3%)	Yes
Oozing telangiectasia	1 (0.6%)	Yes
Duodenal villous atrophy (coeliac disease)	2 (1.3%)	Yes
Barrett's oesophagus	4 (2.6%)	No
Grade B or above oesophagitis	3 (2%)	No
Pangastritis (negative for HP)	3 (2%)	No
Oesophageal stricture	1 (0.6%)	No
Gastric tubular adenoma	1 (0.6%)	No

Cost Saving Economic model

In the group 2 cohort, 88 out of 133 patients had a negative Simtomax test. Based on the 100% sensitivity and NPV of Simtomax, a duodenal biopsy could have been avoided in these 88 patients (66.2%). At the Royal Hallamshire Hospital, the cost of having duodenal biopsies reported (four D2 and one D1 biopsy) is £86, and the price of each Simtomax test kit is £20. The cost saving from avoided biopsies in this cohort would be £7568 (£86x88). After taking into account the cost of using Simtomax on all patients (£20x133=£2660), the overall cost saving in this cohort would be £4908 (£7568-£2660) for the gastroenterology department. This equates to a potential cost saving of £3690 per 100 gastroscopies. At a national level, the difference in the HRG tariff

between a gastroscopy alone and a gastroscopy with duodenal biopsy paid by the clinical commissioners to the trust is £38. This equates to a cost saving of £2514 per 100 gastroscopies for the NHS budget (£38x88/133x100).

5.5: Discussion

This study demonstrated with real life data that the availability of coeliac serology in anaemia prior to gastroscopy was low (33.8%). This result echoes that of an American study conducted by Wiland et al. in 2013 demonstrating that only one third of patients suspected to have coeliac disease had serology available prior to endoscopy (4).

This is the first study showing that Simtomax had 100% sensitivity and NPV in detecting coeliac disease in iron deficient patients. The PLR and NLR were 5.63 and 0 respectively, indicating a negative Simtomax test effectively ruled out coeliac disease in iron deficient patients.

The group 3 duodenal histology analysis revealed no alternative causes for iron deficiency anaemia other than villous atrophy secondary to coeliac disease. With a 100% NPV, taking a duodenal biopsy in patients with a negative Simtomax test would be highly unlikely to yield any other diagnosis for iron deficiency in routine clinical practice, and hence duodenal biopsies could potentially be avoided.

The local cost saving of £3690/100 gastroscopies was based on the coeliac disease prevalence of 19.5% in the group 2 cohort. In a lower prevalence population, the potential cost saving would

be greater. By basing the calculations on the average coeliac disease prevalence of 5% in iron deficient cohorts (144-148), the potential cost saving for the gastroenterology department would be £5826/100 gastroscopies, assuming the same tariff was applied; and £3456/100 gastroscopies would be saved at a national level for the NHS budget. This is excluding a wide range of intangible savings from the positive knock on effects, such as cost savings from not using biopsy pots and forceps, staff time and workload for both the endoscopy and histopathology departments.

The strength of the prospective study (group 2) is that the sensitivities of Simtomax were measured against duodenal histology rather than serology as the reference standard. Furthermore, in order to reduce selection bias, all patients underwent duodenal biopsies irrespective of their serology or Simtomax results. This sets the study apart from the three out of four published Simtomax studies (88-90), where only patients with a positive serology or Simtomax test went on to have duodenal biopsies, which could potentially lead to a positive ascertainment bias and falsely elevated sensitivities. This drawback is also common in previous studies on the sensitivities of coeliac serology as mentioned in the previous chapter.

One of the limitations of the study is the relatively high pre-test probability in group 2 being investigated at our tertiary referral centre for coeliac disease, giving a coeliac disease prevalence of 19.5%. This referral bias may falsely increase the PPV of Simtomax, although the weight of its NPV may be strengthened, which is the focal feature of the point of care test that aims to confidently exclude coeliac disease.

Another limitation is that even though the non-specialist group 3 cohort represented real life data where no other causes for anaemia apart from coeliac disease were found on duodenal histology, it is a relatively small cohort and may not be representative of other populations. For instance, infective causes may be seen in other cohorts, and their prevalence varies from different populations and geographical regions. Two Turkish studies reported a 2% prevalence of Giardiasis found on duodenal biopsies in their cohorts with iron deficiency anaemia (151, 152). In contrast, a German study only found a 0.2% prevalence of Giardiasis on routine duodenal biopsy in 1000 unselected patients attending for a gastroscopy (153), and a study from America had a 0.3% yield of Giardiasis on routine duodenal biopsy in 300 patients presenting with abdominal pain (154). Therefore, the cost saving economic model through biopsy avoidance in iron deficient patients with a negative Simtomax test may not be applicable to populations where parasitic infections are common, as infective diagnoses may be missed.

Of note, in the group 2 tertiary centre iron deficient cohort, there was one case of seronegative villous atrophy secondary to tuberculosis. However, this is a rare cause of seronegative villous atrophy. It must be emphasised that the group 2 cohort does not reflect what is normally seen in routine clinical practice, as the Royal Hallamshire Hospital is a tertiary referral centre for coeliac disease. Apart from coeliac disease and parasitic infections, rarer malabsorptive enteropathies with villous atrophy have been reported in other studies, such as Whipple's disease (155), graft versus host disease (156), common variable immunodeficiency (157, 158), autoimmune enteropathy (159), and olmesartan associated enteropathy (160). The literature has shown that patients with gastrointestinal parasitic infections and other rare enteropathies described above

typically present with significant symptoms such as diarrhoea, abdominal pain and malnutrition, rather than solely with iron deficency anaemia (154, 157-159). Therefore, in iron deficient patients where there is a high index of suspicion for other enteropathies, such as malasborptive symptoms or high risk ethnicities, the threshold for taking duodenal biopsies should be lowered in spite of a negative Simtomax test.

This study demonstrated excellent sensitivity and NPV of Simtomax in iron deficiency, and its performance was comparable to both IgA-EMA and IgA-TTG. Although the results were based on relatively small numbers, they are consistent with two other studies testing Simtomax in high risk groups performed by Benkebil et al. in 2013 (89) (100% sensitivity for coeliac disease in a high risk population) and Bienvenu et al. in 2014 (88) (100% sensitivity and NPV for coeliac disease in IgA deficient children, median age 8.4). Larger studies would be helpful in confirming the diagnostic accuracy of Simtomax in an iron deficient cohort in order to validate the safety of a targeted biopsy approach.

5.6: Conclusion

This is the first study that demonstrated excellent diagnostic accuracy of Simtomax in iron deficiency, which was comparable to conventional serological testing. With a 100% sensitivity and NPV, Simtomax could be used judiciously by clinicians as an effective and cost saving screening test for coeliac disease in the endoscopic setting, by avoiding duodenal biopsies in patients presenting with iron deficiency with a negative Simtomax test (96).

Chapter 6: The role of a point of care test in coeliac disease follow up: predicting histological remission in patients with known coeliac disease on a gluten free diet

6.1: Abstract

Introduction: Mucosal healing is important in coeliac disease for the prevention of complications. However, obtaining duodenal biopsies is invasive, and there is currently no reliable surrogate marker for histological remission in clinical practice. The aim was to assess the role of a point of care test, Simtomax, in detecting persistent villous atrophy in coeliac disease.

Methods: Patients with known coeliac disease attending for a gastroscopy for the assessment of histological remission were prospectively recruited. All patients had Simtomax, IgA-EMA, and IgA-TTG performed, and completed a validated dietary adherence questionnaire. A gastroscopy was performed in all patients, with four biopsies taken from the second part of the duodenum and one from the duodenal bulb. The diagnostic performance of the surrogate markers was measured against duodenal histology as the reference standard.

Results: A total of 217 patients with coeliac disease (70% female, age range 16-83, median age 53) on a gluten free diet (median duration six years) were recruited from 2013-2017. Eighty-five (39.2%) patients had persistent villous atrophy. The sensitivities of Simtomax, IgA-TTG, IgA-EMA and the adherence score in detecting villous atrophy were 67.1%, 44.7%, 37.7% and 24.7% respectively (Simtomax vs IgA-TTG p=0.0005). The combination of Simtomax and adherence score only marginally increased the sensitivity to 70.6% (59.7-80.0%).

Conclusion: The sensitivity of Simtomax was higher than the other surrogate markers in predicting villous atrophy. Simtomax may provide the additional advantage of an immediate objective assessment of mucosal healing at the time of an office based follow-up consultation.

6.2: Introduction

A gluten free diet remains the only treatment for coeliac disease at present. Strict dietary adherence is often challenging given the ubiquity of gluten in Westernised diets and processed foods, with adherence rates reported to vary between 42% and 91% (43, 161, 162). Dietary transgression is the commonest cause for non-responsive coeliac disease (42, 46, 163), which can lead to gastrointestinal and extra-gastrointestinal symptoms, persistent villous atrophy, complications such as osteoporosis and malabsorption, and a worse quality of life (164). A meta-analysis based on over 3000 patients showed that irritable bowel type symptoms were twice as common in patients who were non-adherent compared to those who achieved dietary adherence (pooled odds ratio 2.62, 95% CI 0.75-9.56) (165).

Histological remission is not always achieved in adults, with remission rates ranging from 34%-65% at two years after diagnosis (166-168). This is an important point because persistent villous atrophy may increase the risk of lymphoproliferative malignancies (58) and hip fractures (169). Consequently, the logical approach for disease monitoring would be histological assessment of the duodenum for mucosal healing. However, this method is invasive, costly, and carries risks of complications such as bleeding, perforation and cardiopulmonary complications from sedation (170, 171). Furthermore, there is little consensus for routine follow up biopsy, and the timing of re-biopsy vary among individual practice and national guidelines (35, 36, 172, 173).

There is certainly an unmet need for a reliable surrogate marker for histological remission in

coeliac disease. A myriad of novel markers such as serum intestinal fatty acid-binding protein (I-FABP) levels (174), urinary and faecal gluten immunogenic peptide (175), citrulline (176), faecal fat excretion (177), urinary lactulose-to-mannitol excretion ratios (178), and the maximum concentration of simvastatin in the small intestine (179) have been studied, but none of them are currently used in routine clinical practice.

At present, a combination of dietetic evaluation, symptom assessment and serological titres are used during follow up to determine the necessity for a repeat duodenal biopsy. However, these non-invasive surrogate markers have been shown to correlate poorly with persistent villous atrophy. It is well established that serology is inadequate in detecting ongoing villous atrophy, with sensitivities of IgA-TTG and IgA-EMA reported to be 41-52% and 26-31% respectively (166, 167, 180, 181). Symptoms have also not been shown to associate with histological recovery, where 62% of patients with symptomatic improvement were still found to have persistent villous atrophy (166). Dietary assessment by a specialist dietitian may currently be the optimal method of measuring adherence (182), although the method of assessment is not standardised.

For all these reasons, a simple and reliable method of assessment to measure dietary adherence is needed. A dietary assessment questionnaire was devised by Biagi and colleagues, which contains four simple questions based on the patients' strategy for gluten avoidance rather than assessing the amount of gluten ingested. Biagi et al. concluded that the adherence score identified patients in histological remission, with a PPV of 35.7% and NPV of 86.7% (183). Further

studies are required to validate the utility of this questionnaire.

There is an abundance of studies investigating the performance of different point of care tests in the diagnosis of untreated coeliac disease, with sensitivities of point of care tests (Biocard, Celiac Quick Test and Stick CD1 and 2) reported to be 58-100% (82, 84, 87-89, 121, 123, 125, 184). Conversely, there is a paucity of data examining the role of point of care tests in disease monitoring. Previous studies showed the sensitivities were found to be 78.9% for Simtomax (89), and 77.8% (using whole blood) and 93.5% (using serum) for Celiac Quick Test in measuring dietary adherence in known coeliac disease (184). However, these results should be interpreted with caution as they did not use the presence of villous atrophy as the marker for non-adherence, but instead compared the point of care tests against TTG.

6.3: Methods

Aims:

To evaluate the diagnostic performance of Simtomax, IgA-TTG, IgA-EMA and the adherence questionnaire devised by Biagi et al. in predicting persistent villous atrophy in patients with coeliac disease on a gluten free diet.

Study design and recruitment:

The study took place at the Royal Hallamshire Hospital, Sheffield, U.K., from March 2013-January 2017. Patients with biopsy proven coeliac disease on a gluten free diet who were being assessed for histological remission were prospectively recruited. All patients were concurrently tested with Simtomax, IgA-TTG, IgA-EMA, total IgA levels. A health questionnaire regarding their symptoms (appendix 9) and a dietary adherence questionnaire devised by Biagi et al. (183) (appendix 10) were completed in the endoscopy unit. A gastroscopy with one D1 and four quadrantic D2 biopsies was then performed in all patients.

Inclusion criteria:

Patients with known coeliac disease who were symptomatic, non-adherent to their gluten free diet or those with raised serological titres were recruited. Patients who were asymptomatic, adherent to their diet and with negative serology were not routinely referred for a repeat duodenal biopsy in our centre.

Exclusion criteria:

Gastrointestinal bleeding or evidence of cancer found on endoscopy.

Outcome measures:

The primary outcome was to determine the diagnostic accuracy of Simtomax, IgA-EMA and IgA-TTG in detecting persistent villous atrophy in patients with known coeliac disease.

The secondary outcome was to perform an economic analysis comparing the cost of routine duodenal biopsy for coeliac disease follow up against that of using Simtomax, IgA-EMA and IgA-TTG to target biopsy for those with a positive result.

Statistics:

Sensitivities of the surrogate markers were compared using the McNemar test for correlated proportions.

ROC curves were constructed with paired sensitivities and specificities of each surrogate marker, and the AUC was determined.

Ethics approval:

The study protocol was approved by the Yorkshire and the Humber Research Ethics committee and registered with the local research and development department of Sheffield Teaching Hospital NHS Foundation Trust under the registration number STH15416. Written consent was obtained from all patients. (See appendix 7 for patient information sheet and appendix 8 for consent form)

Economic analysis:

An economic analysis was performed comparing the cost of routine duodenal biopsy against that of using Simtomax, IgA-EMA and IgA-TTG to target biopsy for those with a positive result.

The costs of each item is as follows: Simtomax= £20; TTG= £22.71; EMA= £13.41; combination price of TTG+EMA= £27.96; immunoglobulins= £14.12; processing four D2 and 1 D1 biopsies= £86; formalin biopsy pot= £0.17, biopsy forceps= £4.85; total cost of gastroscopy with duodenal biopsies (£344+ £86+ £0.17x2+ £4.85) = £435.19 (D1 and D2 biopsies were placed in 2 separate pots, hence £0.17x2). Immunoglobulins were routinely measured alongside IgA-TTG and IgA-EMA to identify those with IgA deficiency during follow up, as previous IgA levels may not always be available, especially in cases where the patient was referred from outside our local area. Therefore, the cost of immunoglobulins was taken into account when calculating the cost of using IgA-TTG and IgA-EMA for the purpose of the analysis. Since Simtomax detects both IgA DGP and IgG DGP, there is no need to check immunoglobulins for IgA deficiency when using Simtomax as IgG-DGP would be detected. Therefore, the cost of immunoglobulins was not included in the cost analysis for Simtomax.

6.4: Results

A total of 217 patients with biopsy proven coeliac disease on a gluten free diet were recruited from 2013-2017 (70% female, age range 16-83, median age 53). The median duration of the gluten free diet was six years (76.5 months; range: 6-900 months). Patient characteristics including their gender, race, median duration of gluten free diet in the villous atrophy group and no villous atrophy group are illustrated in table 15. There was no significant difference in symptoms or patient demographics between the two groups.

Table 15: Patient characteristics in the villous atrophy and no villous atrophy groups.

	Villous atrophy group	%	No villous atrophy group	%	P value
Total number of patients	82	n/a	128	n/a	n/a
Male	24	29.3	41	32.0	0.6726
Race: Caucasian	76	92.7	120	93.8	0.7623
Race: Asian	4	4.9	8	6.3	0.6760
Race: Afrocarribean	2	2.4	0	0	0.0758
Median gluten free diet duration	72 months (6 years)	n/a	72 months (6 years)	n/a	n/a
Normal bowel habit	42	51.2	71	55.5	0.5468
Constipation	12	14.6	20	15.6	0.8455
Diarrhea	15	18.3	15	11.7	0.1841
Alternating constipation/ diarrhea	12	14.6	22	17.2	0.7389
Nocturnal diarrhea	7	8.5	11	8.6	0.9885
Urgency	16	19.5	30	23.4	0.5022
Steatorrhea	20	24.4	31	24.2	0.9774
Bloating	37	45.1	69	53.9	0.2142
Weight loss	21	25.6	22	17.2	0.1401
Energy level: normal	29	35.4	43	33.6	0.7918
Energy level: low	53	64.6	85	66.4	0.7918

Table 16: The number of patients for each Marsh grade, and the number and proportion of patients whose surrogate markers correctly identified the presence (Marsh 3a-c histology) or absence (Marsh 0-2 histology) of persistent villous atrophy.

	Marsh 0	**Marsh 1**	**Marsh 2**	**Marsh 0-2**	**Marsh 3a**	**Marsh 3b**	**Marsh 3c**	**Marsh 3a-c**
No. of patients	78	37	17	132	38	24	23	85
Simtomax	48 (61.5%)	23 (62.2%)	7 (41.2%)	78 (59.1%)	21 (55.3%)	21 (87.5%)	15 (65.2%)	57 (67.1%)
IgA-TTG	73 (93.6%)	30 (81.1%)	15 (88.2%)	118 (89.4%)	9 (23.7%)	12 (50.0%)	11 (47.8%)	32 (37.6%)
IgA-EMA	70 (89.8%)	30 (81.1%)	14 (82.4%)	114 (86.5%)	9 (23.7%)	16 (66.7%)	13 (56.5%)	38 (44.7%)
Adherence score	69 (88.5%)	31 (83.8%)	14 (82.4%)	114 (86.4%)	4 (10.5%)	9 (37.5%)	8 (34.8%)	21 (24.7%)

Surrogate markers correctly testing negative for villous atrophy.	Surrogate markers correctly testing positive for villous atrophy.

Eighty-five (39.2%) patients had persistent villous atrophy as defined by Marsh grade 3 histology. Tables 17 and 18 and figures 10 to 13 illustrate the diagnostic performance and the ROC curves for the surrogate markers respectively. The sensitivities of Simtomax, IgA-TTG, IgA-EMA and the adherence score in detecting villous atrophy were 67.1%, 44.7%, 37.7% and 24.7% respectively, where Simtomax had a significantly higher sensitivity than IgA-TTG (Simtomax vs IgA-TTG

p=0.0005). The combination of Simtomax and the adherence score only marginally increased the sensitivity to 70.6% (59.7-80.0%). An economic analysis of using Simtomax, IgA-TTG and IgA-EMA in coeliac disease monitoring is shown in table 18.

Table 17: The diagnostic performance of Simtomax, IgA-TTG, IgA-EMA and the adherence score in detecting persistent villous atrophy, measuring against duodenal histology.

	Sensitivity % (CI)	**Specificity % (CI)**	**PPV % (CI)**	**NPV % (CI)**	**P value (Simtomax vs each surrogate marker)**
Simtomax	67.1 (56.0-76.9)	59.1 (50.2-67.6)	51.4 (45.0-57.6)	73.6 (66.6-79.6)	n/a
IgA-TTG	44.7 (33.9-55.9)	86.4 (79.3-91.7)	67.9 (56.4-77.5)	70.8 (66.5-74.8)	0.0005
IgA-EMA	37.7 (27.4-48.8)	89.4 (82.9-94.1)	69.6 (56.5-80.1)	69.0 (65.1-72.6)	<0.0001
Adherence score	24.7 (16.0-35.3)	86.4 (79.3-91.7)	53.9 (39.8-67.3)	64.0 (60.8-67.2)	<0.0001

Table 18: The diagnostic performance of Simtomax in combination with IgA-TTG, IgA-EMA and the adherence score respectively in detecting persistent villous atrophy, measuring against duodenal histology.

	Sensitivity % (CI)	**Specificity % (CI)**	**PPV % (CI)**	**NPV % (CI)**
Simtomax + IgA-TTG	71.8 (61.0-81.0)	55.5 (46.4-64.0)	50.8 (45.1-56.6)	75.3 (67.7-81.5)
Simtomax + IgA-EMA	71.8 (61.0-81.0)	56.1 (47.2-64.7)	51.3 (45.4-57.1)	75.5 (68.0-81.7)
Simtomax+ adherence score	70.6 (59.7-80.0)	52.3 (43.4-61.0)	48.8 (43.2-54.4)	73.4 (65.7-79.9)

Table 19: Cost analysis of using Simtomax, IgA-TTG, IgA-EMA and duodenal biopsies in coeliac disease monitoring in our cohort.

	Cost for detecting villous atrophy (VA)	**Cost to detect one case of villous atrophy (VA)**	**Cost saving for detecting one case of villous atrophy vs routine re-biopsy**
Duodenal biopsy routinely for all patients (n=217)	£435.19 x217 (total cost of gastroscopy with duodenal biopsies for all patients) = £94436.23	There were 85 cases of VA detected by routine duodenal biopsies. £94436.23/85= £1111.01	n/a
Duodenal biopsy for positive IgA-TTG patients only (n=56)	£22.71 x217 (cost of TTG for all patients) + £14.12 x 217 (cost of immunoglobulins for all patients) + £435.19 x56 (total cost of gastroscopy with duodenal biopsies for positive TTG patients) = £32362.75	There were 38 cases of VA detected by positive TTG. £32362.75/38 = £851.65	£1111.01-£851.65 = £259.36
Duodenal biopsy for positive IgA-EMA patients only (n=46)	£13.41 x217 (cost of EMA for all patients) + £14.12 x217 (cost of immunoglobulins for all patients) + £435.19 x46 (total cost of gastroscopy with duodenal biopsies for positive EMA patients) = £25992.75	There were 29 cases of VA detected by positive EMA. £25992.75/29= £896.30	£1111.01- £896.30 = £214.71
Duodenal biopsy for positive IgA-EMA and/or IgA-TTG patients only (n=56)	£27.96 x217 (cost of combination price of TTG+EMA for all patients) + £14.12 x 217 (cost of immunoglobulins for all patients) + £435.19 x56 (total cost of gastroscopy with duodenal biopsies for positive TTG+/- EMA patients) = £33502	There were 38 cases of VA detected by positive TTG and/or EMA. £33502/38= £881.63	£1111.01- 881.63 = £229.38
Duodenal biopsy for positive Simtomax patients only (n=111)	£20 x217 (cost of Simtomax for all patients) + £435.19 x111 (total cost of gastroscopy with duodenal biopsies for positive Simtomax patients) = £52646.09	There were 57 cases of VA detected by positive Simtomax. £52646.09/57= £923.62	£1111.01- 923.62 = £187.39

Footnote: Simtomax= £20; TTG= £22.71; EMA= £13.41; combination price of TTG+EMA= £27.96; immunoglobulins= £14.12; diagnostic gastroscopy=£344; processing 4 D2 and 1 D1 biopsies= £86; formalin biopsy pot= £0.17, biopsy forceps= £4.85; total cost of gastroscopy with duodenal biopsies (£344+ £86+ £0.17x2+ £4.85) = £435.19 (D1 and D2 biopsies were placed in 2 separate pots, hence £0.17x2)

Figure 10: ROC curve for Simtomax for predicting persistent villous atrophy in known coeliac disease on a gluten free diet. AUC=0.631 (CI: 0.56-0.71).

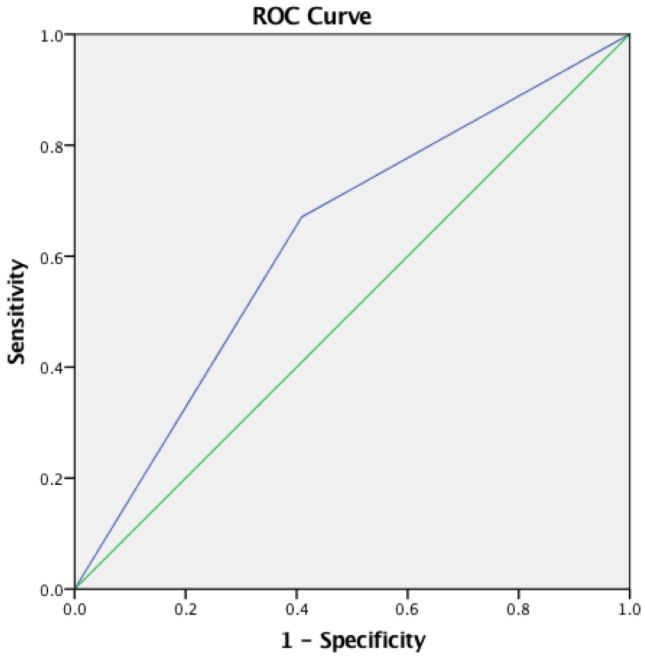


Figure 11: ROC curve for IgA-TTG for predicting persistent villous atrophy in known coeliac disease on a gluten free diet. AUC=0.663 (CI: 0.59-0.74).

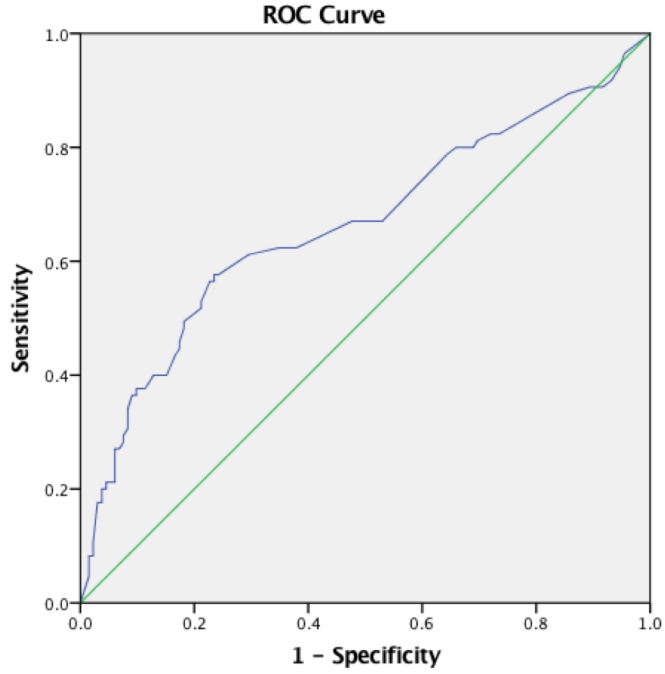


Figure 12: ROC curve for IgA-EMA for predicting persistent villous atrophy in known coeliac disease on a gluten free diet. AUC=0.635 (CI: 0.56-0.71).

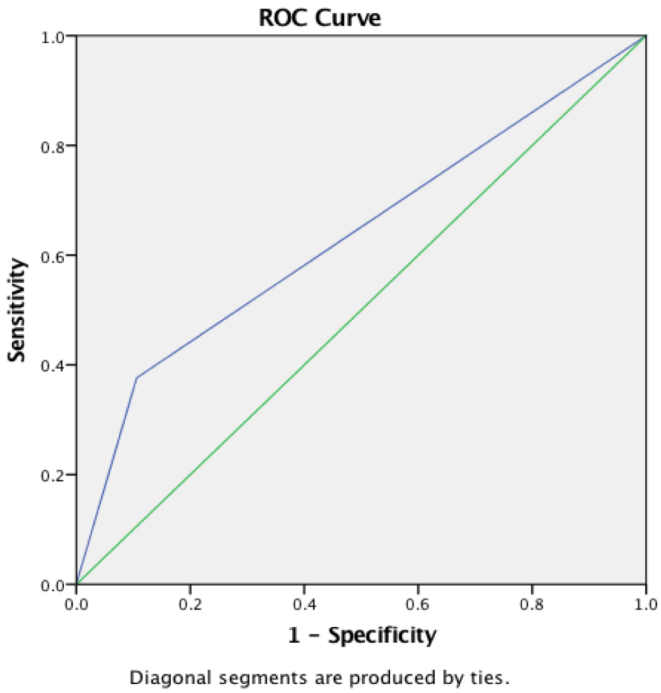
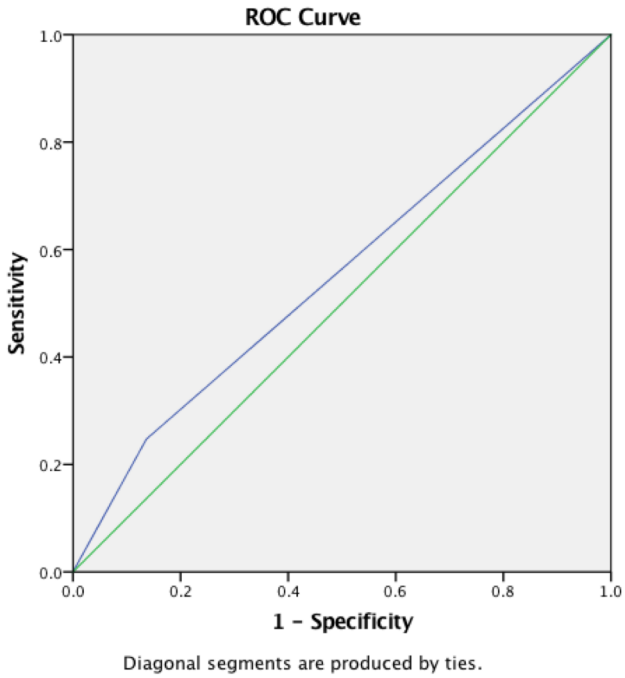


Figure 13: ROC curve for the Biagi adherence score for predicting persistent villous atrophy in known coeliac disease on a gluten free diet. AUC=0.555 (CI: 0.48-0.64).



6.5: Discussion

This is currently the largest study evaluating Simtomax in disease monitoring, showing that Simtomax had a higher sensitivity than conventional serology and the adherence score in detecting ongoing villous atrophy.

One of the strengths of this study is that duodenal biopsies were taken from all patients irrespective of their coeliac antibody or adherence score results, ensuring that false negative cases would be taken into account when calculating the sensitivities and specificities of the surrogate markers. The only other published study investigating the role of Simtomax in disease monitoring was performed by Benkebil et al. (89). The authors tested Simtomax and IgA-TTG in 46 patients with known coeliac disease, but only those with a positive IgA-TTG serology had duodenal biopsies taken. The sensitivity and specificity of Simtomax were reported to be 78.9% and 95.7% respectively. However, these results are unlikely to reflect the actual performance of Simtomax in disease monitoring, as only patients with a positive IgA-TTG were biopsied.

Laboratory DGP serology has been shown in several studies to be useful for disease monitoring in coeliac disease, and appeared to be superior to TTG in this respect (185-188). Spatola et al. showed that IgG-DGP was an effective surrogate marker for histological recovery, with a sensitivity and specificity of 87% and 89% (at a positive threshold of 12U/ml), versus 33% and 100% for IgA-TTG (at a positive threshold of 5U/ml) when tested in 60 patients with known coeliac disease who were strictly adherent, of which 15 (20%) had persistent villous atrophy. ROC curve analysis showed that IgG-DGP substantially outperformed IgA-TTG with an AUC of 0.94

versus 0.61 (186). Similar results were replicated by de Chaisemartin's group subsequently, with a ROC curve analysis demonstrating AUC of 0.817 for IgG-DGP in detecting ongoing villous atrophy (188).

It is not clear why there is such marked difference between the performance of Simtomax and laboratory IgG-DGP serology as previously reported (186, 188). It is conceivable that the threshold for the generation of a positive result in Simtomax is not appropriate for disease monitoring purposes, and the cut off value cannot be adjusted like laboratory serological titres to identify the optimal cut off numerical value. Furthermore, different DGP peptides are used for different DGP assays, thereby possibly giving different levels of sensitivities. It would have been useful to measure laboratory DGP assay alongside Simtomax in this study. However, as previously mentioned, DGP assays are not widely available in the UK, and is not available in our centre. Therefore, DGP assays were not performed, which is a limitation of this study.

Another limitation of this study is the study cohort- the recruited patients were referred for duodenal biopsies to evaluate for histological recovery due to persistence or recurrence of symptoms, positive serology and/or issues with dietary adherence. Although duodenal biopsy is the gold standard for the assessment of disease remission, it is not routinely performed in clinical practice due to the cost and invasiveness of a gastroscopy unless there is a clinical need for it. This means that it is likely that there would be a higher proportion of patients with persistent villous atrophy in our study cohort compared to the general coeliac population, which may mean that the reported diagnostic performance of Simtomax may not be reflective of its use in the

general coeliac population who have no clinical suspicion of disease relapse. Assuming that there will be fewer cases of persistent villous atrophy in the general coeliac cohort, the sensitivities may fall.

Although Simtomax performed significantly better than the other surrogate markers, a sensitivity of 67% is not adequate for it to be used in isolation during follow up, nor is it adequate to replace duodenal biopsy. Combining Simtomax with the dietary adherence score only moderately raised the sensitivity to 70%. Where there is a clinical suspicion of persistent villous atrophy, relying on using Simtomax alone to determine the need for a repeat biopsy may miss cases of villous atrophy, leading to potential complications.

On the other hand, one could look at it from a different perspective: Currently, we do not have a good surrogate marker for mucosal healing at hand. This study demonstrates that this simple point of care test provides a stepwise increase in the diagnostic accuracy of a surrogate marker for persistent villous atrophy to an acceptable level in our search for a better test. Alongside the usual dietetic evaluation and symptom assessment, Simtomax could serve as a useful adjunct to provide instant DGP results during a follow up consultation, not only for the benefit of the clinicians, but also as an immediate feedback for the patients which they highly value. With the use of a point of care test during a clinic consultation, a face to face discussion between the clinician and the patient making a joint decision regarding the need for re-biopsy is made possible.

The low sensitivity of 44.7% for IgA-TTG in detecting persistent villous atrophy in this study is in

line with what has been reported in the literature. For instance, Kaukinen et al. reported the IgA-TTG sensitivity to be 41% (180), and more recently 43.6% by Sharkey's group (167). The even lower sensitivity of 37.7% for IgA-EMA also mirrors the 26% sensitivity reported by Kaukinen et al. (180). The poor association between serological titres and persistent villous atrophy could be explained by the fact that serological testing reflects the adaptive immune response rather than directly measuring intestinal inflammation.

The dietary adherence questionnaire was quick and simple to administer, but its performance in identifying patients with ongoing villous atrophy was disappointing in this study. Recent work by Bannister et al. evaluated this adherence questionnaire and found that the adherence score had a similarly low correlation to villous atrophy, with a sensitivity, specificity, PPV and NPV of 33%, 89%, 13% and 97% respectively (189). The sensitivity and specificity of the adherence score were consistent with our results of 24.7% and 86.4%, however the PPV and NPV were strikingly different from our findings. This could be due to the significantly lower prevalence of persistent villous atrophy in Bannister's paediatric cohort (5.3%) compared to our adult cohort (39.2%), where a low prevalence population could lead to a higher NPV for a diagnostic test.

Indeed, previous follow up studies have demonstrated a slower and more incomplete mucosal healing in adults with coeliac disease treated with a gluten free diet (168, 190-192) compared to children. Potential reasons for the low sensitivity of the adherence questionnaire include reliance on the patient's understanding of what foods contain gluten and how forthcoming they were

regarding their adherence.

An economic analysis was performed for each surrogate marker and the gold standard of duodenal biopsy for the assessment of histological remission (see table 18). Routine re-biopsy was found to be most expensive, costing £94436.23 in our cohort, compared to taking biopsies only in patients with a positive serology or Simtomax, ranging from £25992.75-£52646.09. The detection of persistent villous atrophy differed using different methods, with the gold standard of routine re-biopsy detecting all cases of villous atrophy (n=85), followed by Simtomax (57/85 = 67%), IgA-TTG (38/85 = 44.7%) and IgA-EMA (29/85 = 34%). When taking this into consideration, the cost to detect one case of villous atrophy was most expensive with routine re-biopsy and cheapest with IgA-TTG (£1111.01, £923.62, £896.30 and £851.65 for routine re-biopsy, Simtomax, IgA-EMA and IgA-TTG respectively). Overall, Simtomax was shown to be cheaper than routine re-biopsy although slightly more expensive than conventional serology. Nevertheless, it is important for clinicians to consider not only the cost, but also the efficacy of the surrogate markers and the invasiveness of a gastroscopy as a whole when deciding which method should be used for disease monitoring in coeliac disease.

6.6: Conclusion

To conclude, this study showed for the first time that the commercially available DGP based point of care test, Simtomax, had a superior sensitivity in detecting persistent villous atrophy in patients with known coeliac disease, compared to the adherence score and conventional coeliac

serology (IgA-TTG and IgA-EMA) which are routinely used for disease monitoring at present. Simtomax could help streamline the follow up process by providing DGP results during the consultation, and facilitate the joint decision making between the clinician and the patient regarding the onward management plan such as the necessity of follow up duodenal biopsy.

Chapter 7: Summary of key findings, recommendations for future research and conclusions

7.1 Summary of key findings and recommendations for future research

In this body of research that I have undertaken, I have assessed the diagnostic accuracy and utility of Simtomax in various clinical settings, including case finding in primary care, secondary care, pre-endoscopy and coeliac disease assessment at follow up. Despite the early promising results for Simtomax with the initial published reports, this was not borne out in the studies which I undertook. For example, point of care testing in community pharmacies produced a low yield for case detection, even though the sensitivity of Simtomax was in fact comparable to TTG serology. The low yield in primary care is unlikely to be due to sensitivity issues. A previous point of care test case finding study by Urwin et al. based in 15 pharmacies across England (97) also had a similar Simtomax positive rate, indicating that the low yield in primary care is probably not confined to our local area but beyond. Disappointingly, the limitation of Simtomax lies in its low specificity. On balance, the high false positive rate outweighs the advantage of providing rapid and sensitive antibody results in real time, due to the high number patients who would be subjected to undergo unnecessary investigations. Given the above findings, I consider that Simtomax cannot be recommended as a suitable test for general case finding in both primary and secondary care in the NHS. Nonetheless, it is important to point out that point of care testing for coeliac disease may be of value in a different healthcare system. For instance, two case finding studies based in Brazil found that the use of a point of care test for coeliac disease was far more effective and cost saving than conventional serology, due to limited facilities and the high cost of laboratory based tests in a low income country (193, 194).

It is intriguing that laboratory DGP serology has been shown to have a higher specificity than

sensitivity, whereas the opposite was observed for Simtomax. A systematic review reported a pooled sensitivity, specificity, PPV and NPV of combined IgA/IgG-DGP serology to be 87-98.3% (median 95.2%), 98.8-100% (median 96%), 98.3-100% (median 99%) and 97-98.8% (median 97.9%) respectively (195). One should bear in mind that these values are much higher than our data as the coeliac disease prevalence in these studies were very high, ranging from 39.1-44.3%, compared to 4% in our cohort in chapter 4. Nevertheless, it is not clear why the specificity and PPV of Simtomax from chapter 4 are substantially lower than the published data. Diagnostic performance is considerably influenced by a wide range of factors. Methodological disparities such as study design, inclusion criteria, disease prevalence, reference standard and disease spectrum (severity of villous atrophy) are well known to affect diagnostic performance. Additionally, specific to Simtomax, the difference in the method of DGP testing (lateral flow immunochromatography vs ELISA) also plays a role in the incongruent results to DGP serology. A combination of two specific DGP peptides which were shown to provide superior performance were selected for Simtomax based on a study by Schwertz et al. (92). These DGP peptides may differ from those used for laboratory DGP serology that were studied (Quanta Lite Gliadin II IgG and IgA; Inova Diagnostics, San Diego, CA, USA) and hence giving different results. Furthermore, there is a mechanical filter in the Simtomax device that separates whole blood by removing red blood cells and nucleated cells to allow the serum proteins to be analysed using antigen direct sandwich assay technique, which is very similar to ELISA. It is plausible that the filtering process may cause a low degree of haemolysis which would adversely affect the plasma flow due to increased turbidity, and thereby increasing background noise, hence affecting precision of the test. Moreover, it is also conceivable that the wicking rate and adsorption control of the

nitrocellulose membrane of the device where the assay binding occurs may play a part in influencing sensitivities.

As for the assessment of disease remission, even though Simtomax was the most sensitive marker for predicting persistent villous atrophy compared to serology and a dietary adherence questionnaire, its sensitivity of 67% is still considerably inadequate to be solely relied upon, and cannot replace repeat duodenal biopsies. For now, Simtomax may be used as an adjunct in follow up clinics to help determine the need for repeat duodenal biopsies, but I think it will soon be superseded by other more promising surrogate markers such as gluten immunogenic peptides.

Undoubtedly, there is a demand for point of care tests in clinical practice, and a good example is point of care tests for INR which has been well established in its role in both primary and secondary care in the last decade. The successful implementation of INR point of care testing by healthcare professionals was based on acceptable diagnostic performance compared to laboratory INR testing. A systematic review in 2012 analysing 20 studies found the correlation coefficient of CoaguCheck XS varied from 0.81 to 0.98, whilst that of INRatio and ProTime varied between 0.73 and 0.95 when compared to laboratory INR as the reference standard (196). However, uncertain cost effectiveness based on conflicting results from previous studies (136) meant that INR point of care testing has not been approved for routine patient use for self-monitoring. Deriving from this example, I have demonstrated that Simtomax not only had comparable diagnostic accuracy to conventional serology, but also cost effectiveness when used in the pre-endoscopy setting for patients with iron deficiency anaemia. Therefore, I believe the

area of potential for Simtomax is within this setting. With the 100% NPV, Simtomax could be used as a cost saving screening tool through biopsy avoidance in a cohort where routine duodenal biopsy is common practice.

Despite this, I think there are barriers to overcome at multiple levels before this could be implemented within the NHS. First of all, the study sample size was relatively small (chapter 5) (n=144), therefore the results will have to be rigorously validated with a larger or multicentre study before being approved by the NHS. Whilst INR POCT has clinician 'buy in', I do not think (based on my own experience of presenting the data widely) that Gastroenterology doctors regard this coeliac POCT in the same way. One of the reasons I would suggest is that this is due to the poor specificity of the test.

Finances and time pressures also play a negative role from the endoscopy directorate's perspective. Using Simtomax in endoscopy involves training nurses to perform the test. Nurses may be reluctant to take it on as it consumes more time and effort during the pre-assessment process, where time constraint is commonplace within the existing healthcare system. Additionally, having to pay for the Simtomax kits upfront before any potential longer term cost savings are apparent may also deter the directorate from taking the risk to invest in this economic model. This cost saving model was based on routine duodenal biopsy for anaemic patients, but biopsy practice may differ in other hospitals, and this could also be influenced by their pre-endoscopy availability of coeliac serology results. Although we have demonstrated that only 30% of patients with anaemia had serology performed prior to endoscopy in four UK hospitals, the

pre-endoscopy serology availability will vary amongst different hospitals. If we consider HRG tariffs, a gastroscopy with biopsy is remunerated at a higher level than a gastroscopy without biopsy. So although Simtomax may reduce biopsy rates, the local NHS manager may view that this results in paying for the cost of the test as well as reducing revenue for their directorate.

Moreover, the endoscopists may disregard the Simtomax test results, whether it be due to clinical reasons, hesitance in relying on the accuracy of Simtomax, or inertia to change practice, and hence the cost saving potential in reality may differ from the estimated calculations I made. On the other hand, from the histopathology department's standpoint, even though biopsy avoidance would reduce their workload, they may see this as a reduction of revenue for the department. The histopathology department is funded on units of work irrespective of the complexity of the work. Controversially, looking at it from a human factor or work intensity angle, one may prefer having to process and report straightforward normal duodenal biopsies to evaluating more complex abnormal histological samples as the bulk of their daily work. These potential obstacles may arise and have to be overcome in order to successfully implement the use of Simtomax in practice.

Overall, Simtomax fits the criteria for being an excellent test in many ways, including comparable sensitivity to TTG serology, convenience, rapid result availability and high user satisfaction. Unfortunately, its low specificity presents a major setback and needs to be addressed. A recent study reported on a novel gliadin peptide known as 8-mer gliadin peptide that is released by gliadin degrading metalloproteinases in the duodenal mucosa found only in patients with coeliac

disease. The specificity of this peptide antibody was found to be 98.8% (197). Further research such as this may help to enhance the diagnostic performance of a point of care test for coeliac disease by integrating highly accurate peptide sequences in a lateral flow format.

7.2 Conclusion

Simtomax has been shown to have comparable sensitivities and practical advantages to serology. However, its low specificity represents a major limitation, as its use in a general cohort of symptomatic patients may lead to a rise in unnecessary investigations. In regards to predicting histological remission, Simtomax had a significantly higher sensitivity compared to TTG serology. However, its sensitivity is not reliable enough to replace duodenal biopsies. I believe Simtomax may be best suited to be used in the pre-endoscopy setting for anaemic patients in hospitals that would derive cost saving benefits from it.

References

1. West J, Logan RF, Hill PG, Lloyd A, Lewis S, Hubbard R, et al. Seroprevalence, correlates, and characteristics of undetected coeliac disease in England. Gut. 2003;52(7):960-5.
2. Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, et al. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. Arch Intern Med. 2003;163(3):286-92.
3. West J, Fleming KM, Tata LJ, Card TR, Crooks CJ. Incidence and prevalence of celiac disease and dermatitis herpetiformis in the UK over two decades: population-based study. Am J Gastroenterol. 2014;109(5):757-68.
4. Wiland HO, Henricks WH, Daly TM. Limited utilization of serologic testing in patients undergoing duodenal biopsy for celiac disease. BMC Gastroenterol. 2013;13:156.
5. Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, et al. Prevalence of celiac disease in at- risk and not-at- risk groups in the United States: a large multicenter study. Archives of internal medicine. 2003;163(3):286.
6. Ludvigsson JF, Rubio-Tapia A, van Dyke CT, Melton LJ, 3rd, Zinsmeister AR, Lahr BD, et al. Increasing incidence of celiac disease in a North American population. Am J Gastroenterol. 2013;108(5):818-24.
7. Sollid LM, Thorsby E. HLA susceptibility genes in celiac disease: genetic mapping and role in pathogenesis. Gastroenterology. 1993;105(3):910-22.
8. SV H. The value of the banana in the treatment of celiac disease. Archives of Pediatrics & Adolescent Medicine. 1924;28:421.
9. Van de Kamer JH. An investigation into the injurious constituents of wheat in connection with their action on patients with coeliac disease. Acta Paediatrica. 1953;42:223-31.
10. Ludvigsson JF, Leffler DA, Bai JC, Biagi F, Fasano A, Green PH, et al. The Oslo definitions for coeliac disease and related terms. Gut. 2013;62(1):43-52.
11. Campbell CB, Roberts RK, Cowen AE. The changing clinical presentation of coeliac disease in adults. Med J Aust. 1977;1(4):89-93.
12. Logan RFA, Tucker G, Rifkind EA, Heading RC, Ferguson A. Changes in clinical features of coeliac disease in adults in Edinburgh and the Lothians 1960- 79. British Medical Journal. 1983;286(6359):95-7.
13. Maki M, Kallonen K, Lahdeaho ML, Visakorpi JK. Changing pattern of childhood coeliac disease in Finland. Acta Paediatr Scand. 1988;77(3):408-12.
14. Sanders DS, Hurlstone DP, Stokes RO, Rashid F, Milford-Ward A, Hadjivassiliou M, et al. Changing face of adult coeliac disease: experience of a single university hospital in South Yorkshire. Postgrad Med J. 2002;78(915):31-3.
15. Green P, Lebwohl B, Greywoode R. Celiac disease. J Allergy Clin Immunol2015. p. 1099-106.
16. Alarida K, Harown J, Ahmaida A, Marinelli L, Venturini C, Kodermaz G, et al. Coeliac disease in Libyan children: A screening study based on the rapid determination of anti- transglutaminase antibodies. Digestive and Liver Disease. 2011;43(9):688-91.
17. Wang XQ, Liu W, Xu CD, Mei H, Gao Y, Peng HM, et al. Celiac disease in children with diarrhea in 4 cities in China. J Pediatr Gastroenterol Nutr. 2011;53(4):368-70.
18. Gupta R, Reddy DN, Makharia GK, Sood A, Ramakrishna BS, Yachha SK, et al. Indian task force for celiac disease: current status. 2009. p. 6028.
19. Buket D, Sinan S, Bilkay B, Arzu E, Odul E, Aysegul B, et al. Prevalence of Celiac Disease in Healthy Turkish School Children. The American Journal of Gastroenterology. 2011;106(8):1512.
20. Beitnes AR, Vikskjold FB, Johannesdottir GB, Perminow G, Olbjrn C, Andersen SN, et al. Symptoms and Mucosal Changes Stable During Rapid Increase of Pediatric Celiac Disease in Norway. J Pediatr Gastroenterol Nutr. 2016.

21. Lohi S, Mustalahti K, Kaukinen K, Laurila K, Collin P, Rissanen H, et al. Increasing prevalence of coeliac disease over time. Aliment Pharmacol Ther. 2007;26(9):1217-25.
22. Okada H, Kuhn C, Feillet H, Bach JF. The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. Clin Exp Immunol. 2010;160(1):1-9.
23. Singh P, Arora S, Singh A, Strand TA, Makharia GK. Su1176 Prevalence of Celiac Disease in Asia: A Systematic Review and Meta- Analysis. 2016. p. S487-S.
24. Mora M, Litwin N, Toca MdC, Azcona MI, Solís Neffa R, Battiston F, et al. Prevalence of celiac disease: multicentric trial among pediatric population from five urban districts in Argentina. Archivos argentinos de pediatría. 2012;110(6):490-6.
25. Yap TW-C, Loke M-F, Vadivelu J, Chan W-K, Leow AH-R, Azmi AN, et al. Prevalence of serum celiac antibodies in a multiracial asian population - A first study in the Young Asian adult population of Malaysia. PLoS ONE. 2015;10(3).
26. Al Hatlani MM. Prevalence of celiac disease among symptom- free children from the Eastern Province of Saudi Arabia. Saudi Journal of Gastroenterology. 2015;21(6):367-71.
27. Hadithi M, Von Blomberg BME, Crusius JBA, Bloemena E, Kostense PJ, Meijer JWR, et al. Accuracy of serologic tests and HLA- DQ typing for diagnosing celiac disease. Annals of internal medicine. 2007;147(5):294.
28. Schuppan D, Junker Y, Barisani D. Celiac Disease: From Pathogenesis to Novel Therapies. Gastroenterology. 2009;137(6):1912-33.
29. Wieser H. Relation between gliadin structure and coeliac toxicity. Acta Paediatr Suppl. 1996;412:3-9.
30. Fasano A. Zonulin, regulation of tight junctions, and autoimmune diseases. Annals of the New York Academy of Sciences. 2012;12581(1):25-33.
31. Dieterich W, Tobias E, Michael B, Peter D, Umberto V, Ernst Otto R, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nature Medicine. 1997;3(7):797.
32. Rescigno M, Di Sabatino A. Dendritic cells in intestinal homeostasis and disease. J Clin Invest2009. p. 2441-50.
33. Koning F, Schuppan D, Cerf-Bensussan N, Sollid LM. Pathomechanisms in celiac disease. Best Practice & Research Clinical Gastroenterology. 2005;19(3):373-87.
34. Gray AM, Papanicolas IN. Impact of symptoms on quality of life before and after diagnosis of coeliac disease: results from a UK population survey. BMC Health Services Research. 2010;10:105-.
35. Ludvigsson JF, Bai JC, Biagi F, Card TR, Ciacci C, Ciclitira PJ, et al. Diagnosis and management of adult coeliac disease: guidelines from the British Society of Gastroenterology. Gut. 2014;63(8):1210-28.
36. Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA, American College of G. ACG clinical guidelines: diagnosis and management of celiac disease. Am J Gastroenterol. 2013;108(5):656-76; quiz 77.
37. West J, Logan RFA, Card TR, Smith C, Hubbard R. Fracture risk in people with celiac disease: a population- based cohort study. Gastroenterology. 2003;125(2):429-36.
38. Ludvigsson JF, Montgomery SM, Ekbom A. Celiac disease and risk of adverse fetal outcome: a population-based cohort study. Gastroenterology. 2005;129(2):454-63.
39. Rostami K, Steegers EA, Wong WY, Braat DD, Steegers-Theunissen RP. Coeliac disease and reproductive disorders: a neglected association. Eur J Obstet Gynecol Reprod Biol. 2001;96(2):146-9.
40. Pynnonen PA, Isometsa ET, Verkasalo MA, Kahkonen SA, Sipila I, Savilahti E, et al. Gluten-free diet may alleviate depressive and behavioural symptoms in adolescents with coeliac disease: a prospective follow-up case-series study. BMC Psychiatry. 2005;5:14.
41. Ludvigsson JF, Reutfors J, Osby U, Ekbom A, Montgomery SM. Coeliac disease and risk of mood disorders--a general population-based cohort study. J Affect Disord. 2007;99(1-3):117-26.

42. Dewar DH, Donnelly SC, McLaughlin SD, Johnson MW, Ellis HJ, Ciclitira PJ. Celiac disease: management of persistent symptoms in patients on a gluten-free diet. World J Gastroenterol. 2012;18(12):1348-56.
43. Hall NJ, Rubin G, Charnock A. Systematic review: adherence to a gluten-free diet in adult patients with coeliac disease. Aliment Pharmacol Ther. 2009;30(4):315-30.
44. Sainsbury K, Mullan B. Measuring beliefs about gluten free diet adherence in adult coeliac disease using the theory of planned behaviour. Appetite. 2011;56(2):476-83.
45. Biagi F, Corazza GR. Defining gluten refractory enteropathy. Eur J Gastroenterol Hepatol. 2001;13(5):561-5.
46. Abdulkarim AS, Burgart LJ, See J, Murray JA. Etiology of nonresponsive celiac disease: results of a systematic approach. Am J Gastroenterol. 2002;97(8):2016-21.
47. West J. Celiac disease and its complications: a time traveller's perspective. Gastroenterology. 2009;136(1):32-4.
48. Roshan B, Leffler DA, Jamma S, Dennis M, Sheth S, Falchuk K, et al. The incidence and clinical spectrum of refractory celiac disease in a north american referral center. Am J Gastroenterol. 2011;106(5):923-8.
49. Rubio-Tapia A, Kelly DG, Lahr BD, Dogan A, Wu TT, Murray JA. Clinical staging and survival in refractory celiac disease: a single center experience. Gastroenterology. 2009;136(1):99-107; quiz 352-3.
50. Al-Toma A, Verbeek WH, Hadithi M, von Blomberg BM, Mulder CJ. Survival in refractory coeliac disease and enteropathy-associated T-cell lymphoma: retrospective evaluation of single-centre experience. Gut. 2007;56(10):1373-8.
51. Gale J, Simmonds PD, Mead GM, Sweetenham JW, Wright DH. Enteropathy-type intestinal T-cell lymphoma: clinical features and treatment of 31 patients in a single center. J Clin Oncol. 2000;18(4):795-803.
52. Malamut G, Afchain P, Verkarre V, Lecomte T, Amiot A, Damotte D, et al. Presentation and long-term follow-up of refractory celiac disease: comparison of type I with type II. Gastroenterology. 2009;136(1):81-90.
53. Nijeboer P, de Baaij LR, Visser O, Witte BI, Cillessen SA, Mulder CJ, et al. Treatment response in enteropathy associated T-cell lymphoma; survival in a large multicenter cohort. Am J Hematol. 2015;90(6):493-8.
54. Rubio–Tapia A, Kyle RA, Kaplan EL, Johnson DR, Page W, Erdtmann F, et al. Increased Prevalence and Mortality in Undiagnosed Celiac Disease. Gastroenterology. 2009;137(1):88-93.
55. Daum S, Ipczynski R, Heine B, Schulzke JD, Zeitz M, Ullrich R. Therapy with budesonide in patients with refractory sprue. Digestion. 2006;73(1):60-8.
56. Goerres MS, Meijer JW, Wahab PJ, Kerckhaert JA, Groenen PJ, Van Krieken JH, et al. Azathioprine and prednisone combination therapy in refractory coeliac disease. Aliment Pharmacol Ther. 2003;18(5):487-94.
57. Al-Toma A, Goerres MS, Meijer JW, von Blomberg BM, Wahab PJ, Kerckhaert JA, et al. Cladribine therapy in refractory celiac disease with aberrant T cells. Clin Gastroenterol Hepatol. 2006;4(11):1322-7; quiz 00.
58. Lebwohl B, Granath F, Ekbom A, Smedby KE, Murray JA, Neugut AI, et al. Mucosal healing and risk for lymphoproliferative malignancy in celiac disease: a population-based cohort study. Annals of internal medicine. 2013;159(3):169.
59. Elfström P, Granath F, Ekström Smedby K, Montgomery SM, Askling J, Ekbom A, et al. Risk of Lymphoproliferative Malignancy in Relation to Small Intestinal Histopathology Among Patients With Celiac Disease. Journal of the National Cancer Institute. 2011;103(5):436-44.
60. Downey L, Houten R, Murch S, Longson D. Recognition, assessment, and management of coeliac disease: summary of updated NICE guidance. Bmj. 2015;351:h4513.

61. Grodzinsky E, Jansson G, Skogh T, Stenhammar L, Fälth-Magnusson K. Anti-endomysium and anti-gliadin antibodies as serological markers for coeliac disease in childhood: a clinical study to develop a practical routine. Acta Paediatr. 1995;84(3):294-8.
62. van der Windt DA, Jellema P, Mulder CJ, Kneepkens CM, van der Horst HE. Diagnostic testing for celiac disease among patients with abdominal symptoms: a systematic review. JAMA. 2010;303(17):1738-46.
63. Vitoria JC, Arrieta A, Arranz C, Ayesta A, Sojo A, Maruri N, et al. Antibodies to gliadin, endomysium, and tissue transglutaminase for the diagnosis of celiac disease. J Pediatr Gastroenterol Nutr. 1999;29(5):571-4.
64. Giersiepen K, Lelgemann M, Stuhldreher N, Ronfani L, Husby S, Koletzko S, et al. Accuracy of diagnostic antibody tests for coeliac disease in children: summary of an evidence report. J Pediatr Gastroenterol Nutr. 2012;54(2):229-41.
65. Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, et al. European society for pediatric gastroenterology, hepatology, and nutrition guidelines for the diagnosis of coeliac disease. Revista Portuguesa de Imunoalergologia. 2012;20(3):227-8.
66. Natalia EC, Rohini RV, Thimmaiah GT, Alberto R-T, Joseph AM, Javier V, et al. Prevalence of Abnormal Liver Function Tests in Celiac Disease and the Effect of a Gluten- Free Diet in the US Population. The American Journal of Gastroenterology. 2015;110(8):1216.
67. Ludvigsson JF, Olen O, Bell M, Ekbom A, Montgomery SM. Coeliac disease and risk of sepsis. Gut. 2008;57(8):1074-80.
68. Haines ML, Anderson RP, Gibson PR. Systematic review: the evidence base for long- term management of coeliac disease. Oxford, UK2008. p. 1042-66.
69. Paterson BM, Lammers KM, Arrieta MC, Fasano A, Meddings JB. The safety, tolerance, pharmacokinetic and pharmacodynamic effects of single doses of AT-1001 in coeliac disease subjects: a proof of concept study. Aliment Pharmacol Ther. 2007;26(5):757-66.
70. Leffler DA, Kelly CP, Abdallah HZ, Colatrella AM, Harris LA, Leon F, et al. A randomized, double-blind study of larazotide acetate to prevent the activation of celiac disease during gluten challenge. Am J Gastroenterol. 2012;107(10):1554-62.
71. Kelly CP, Green PH, Murray JA, Dimarino A, Colatrella A, Leffler DA, et al. Larazotide acetate in patients with coeliac disease undergoing a gluten challenge: a randomised placebo-controlled study. Aliment Pharmacol Ther. 2013;37(2):252-62.
72. Tack GJ, van de Water JM, Bruins MJ, Kooy-Winkelaar EM, van Bergen J, Bonnet P, et al. Consumption of gluten with gluten-degrading enzyme by celiac patients: a pilot-study. World J Gastroenterol. 2013;19(35):5837-47.
73. Siegel M, Garber ME, Spencer AG, Botwick W, Kumar P, Williams RN, et al. Safety, tolerability, and activity of ALV003: results from two phase 1 single, escalating-dose clinical trials. Dig Dis Sci. 2012;57(2):440-50.
74. Lahdeaho ML, Kaukinen K, Laurila K, Vuotikka P, Koivurova OP, Karja-Lahdensuu T, et al. Glutenase ALV003 attenuates gluten-induced mucosal injury in patients with celiac disease. Gastroenterology. 2014;146(7):1649-58.
75. Daveson AJM, Ee HC, Andrews JM, King T, Goldstein KE, Dzuris JL, et al. Epitope-Specific Immunotherapy Targeting CD4-Positive T Cells in Celiac Disease: Safety, Pharmacokinetics, and Effects on Intestinal Histology and Plasma Cytokines with Escalating Dose Regimens of Nexvax2 in a Randomized, Double-Blind, Placebo-Controlled Phase 1 Study. EBioMedicine. 2017;26:78-90.
76. Mizen L. Simtomax®: a new screening tool for coeliac diseaseSimtomax®: a new screening tool for coeliac disease. British Journal of Healthcare Management. 2012;18(1):27-32.
77. Bittolo M, Not T, Perticarari S, Cauci S, Graziosi G, Marzari R. A dot immunobinding assay to detect anti-alpha-gliadin antibodies in celiac disease. J Pediatr Gastroenterol Nutr. 1990;11(3):337-41.

78. Not T, Ventura A, Peticarari S, Basile S, Torre G, Dragovic D. A new, rapid, noninvasive screening test for celiac disease. J Pediatr. 1993;123(3):425-7.
79. Garrote JA, Sorell L, Alfonso P, Acevedo B, Ortigosa L, Ribes-Koninckx C, et al. A novel visual immunoassay for coeliac disease screening. Eur J Clin Invest. 1999;29(8):697-9.
80. Baldas V, Tommasini A, Trevisiol C, Berti I, Fasano A, Sblattero D, et al. Development of a novel rapid non-invasive screening test for coeliac disease. Gut. 2000;47(5):628-31.
81. Sorell L, Garrote JA, Acevedo B, Arranz E. One-step immunochromatographic assay for screening of coeliac disease. Lancet. 2002;359(9310):945-6.
82. Raivio T, Kaukinen K, Nemes E, Laurila K, Collin P, Kovács JB, et al. Self transglutaminase-based rapid coeliac disease antibody detection by a lateral flow method. Aliment Pharmacol Ther. 2006;24(1):147-54.
83. Raivio T, Korponay-Szabó IR, Paajanen T, Ashorn M, Iltanen S, Collin P, et al. Comparison of a novel whole blood transglutaminase-based ELISA with a whole blood rapid antibody test and established conventional serological celiac disease assays. J Pediatr Gastroenterol Nutr. 2008;47(5):562-7.
84. Mooney PD, Kurien M, Evans KE, Chalkiadakis I, Hale MF, Kannan MZ, et al. Point-of-care testing for celiac disease has a low sensitivity in endoscopy. Gastrointest Endosc. 2014;80(3):456-62.
85. Singh P, Wadhwa N, Chaturvedi MK, Bhatia V, Saini S, Tandon N, et al. Validation of point-of-care testing for coeliac disease in children in a tertiary hospital in north India. Arch Dis Child. 2014;99(11):1004-8.
86. Raivio T, Korponay-Szabó I, Collin P, Laurila K, Huhtala H, Kaartinen T, et al. Performance of a new rapid whole blood coeliac test in adult patients with low prevalence of endomysial antibodies. Dig Liver Dis. 2007;39(12):1057-63.
87. Mooney PD, Wong SH, Johnston AJ, Kurien M, Avgerinos A, Sanders DS. Increased Detection of Celiac Disease With Measurement of Deamidated Gliadin Peptide Antibody Before Endoscopy. Clin Gastroenterol Hepatol. 2015;13(7):1278-84.e1.
88. Bienvenu F, Anghel SI, Besson Duvanel C, Guillemaud J, Garnier L, Renosi F, et al. Early diagnosis of celiac disease in IgA deficient children: contribution of a point-of-care test. BMC Gastroenterol. 2014;14:186.
89. Benkebil F, Combescure C, Anghel SI, Besson Duvanel C, Schäppi MG. Diagnostic accuracy of a new point-of-care screening assay for celiac disease. World J Gastroenterol. 2013;19(31):5111-7.
90. Bienvenu F, Besson Duvanel C, Seignovert C, Rouzaire P, Lachaux A, Bienvenu J. Evaluation of a point-of-care test based on deamidated gliadin peptides for celiac disease screening in a large pediatric population. Eur J Gastroenterol Hepatol. 2012;24(12):1418-23.
91. Molberg O, McAdam S, Korner R, Quarsten H, Kristiansen C, Madsen L, et al. Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease (vol 4, pg 713, 1998). Nature Medicine. 1998;4(8):974-.
92. Schwertz E, Kahlenberg F, Sack U, Richter T, Stern M, Conrad K, et al. Serologic assay based on gliadin-related nonapeptides as a highly sensitive and specific diagnostic aid in celiac disease. Clin Chem. 2004;50(12):2370-5.
93. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. Eur J Gastroenterol Hepatol. 1999;11(10):1185-94.
94. Sanders DS, Patel D, Stephenson TJ, Ward AM, McCloskey EV, Hadjivassiliou M, et al. A primary care cross-sectional study of undiagnosed adult coeliac disease. Eur J Gastroenterol Hepatol. 2003;15(4):407-13.
95. Hin H, Bird G, Fisher P, Mahy N, Jewell D. Coeliac disease in primary care: case finding study. BMJ. 1999;318(7177):164-7.

96. Lau MS, Mooney P, White W, Appleby V, Moreea S, Haythem I, et al. 'Pre-endoscopy point of care test (Simtomax- IgA/IgG-Deamidated Gliadin Peptide) for coeliac disease in iron deficiency anaemia: diagnostic accuracy and a cost saving economic model'. BMC Gastroenterol. 2016;16:115.
97. Urwin H, Wright D, Twigg M, McGough N. Early recognition of coeliac disease through community pharmacies: a proof of concept study. Int J Clin Pharm. 2016;38(5):1294-300.
98. Hernandez-Sampelayo P, Seoane M, Oltra L, Marin L, Torrejon A, Vera MI, et al. Contribution of nurses to the quality of care in management of inflammatory bowel disease: a synthesis of the evidence. J Crohns Colitis. 2010;4(6):611-22.
99. Mustalahti K, Catassi C, Reunanen A, Fabiani E, Heier M, McMillan S, et al. The prevalence of celiac disease in Europe: results of a centralized, international mass screening project. Ann Med. 2010;42(8):587-95.
100. Rubio-Tapia A, Ludvigsson JF, Brantner TL, Murray JA, Everhart JE. The prevalence of celiac disease in the United States. Am J Gastroenterol. 2012;107(10):1538-44; quiz 7, 45.
101. Hill I, Fasano A, Schwartz R, Counts D, Glock M, Horvath K. The prevalence of celiac disease in at-risk groups of children in the United States. J Pediatr. 2000;136(1):86-90.
102. Singh P, Arora S, Singh A, Strand TA, Makharia GK. Prevalence of celiac disease in Asia: A systematic review and meta-analysis. J Gastroenterol Hepatol. 2016;31(6):1095-101.
103. Yap TW, Chan WK, Leow AH, Azmi AN, Loke MF, Vadivelu J, et al. Prevalence of serum celiac antibodies in a multiracial Asian population--a first study in the young Asian adult population of Malaysia. PLoS One. 2015;10(3):e0121908.
104. Makharia GK, Mulder CJ, Goh KL, Ahuja V, Bai JC, Catassi C, et al. Issues associated with the emergence of coeliac disease in the Asia-Pacific region: a working party report of the World Gastroenterology Organization and the Asian Pacific Association of Gastroenterology. J Gastroenterol Hepatol. 2014;29(4):666-77.
105. Talley NJ, Walker MM. Celiac Disease and Nonceliac Gluten or Wheat Sensitivity: The Risks and Benefits of Diagnosis. JAMA Internal Medicine. 2017;177(5):615-6.
106. Leonard MM, Sapone A, Catassi C, Fasano A. Celiac Disease and Nonceliac Gluten Sensitivity: A Review. JAMA. 2017;318(7):647-56.
107. Sapone A, Bai JC, Ciacci C, Dolinsek J, Green PH, Hadjivassiliou M, et al. Spectrum of gluten-related disorders: consensus on new nomenclature and classification. BMC Med. 2012;10:13.
108. Catassi C, Elli L, Bonaz B, Bouma G, Carroccio A, Castillejo G, et al. Diagnosis of Non-Celiac Gluten Sensitivity (NCGS): The Salerno Experts' Criteria. Nutrients. 2015;7(6):4966-77.
109. Aziz I, Lewis NR, Hadjivassiliou M, Winfield SN, Rugg N, Kelsall A, et al. A UK study assessing the population prevalence of self-reported gluten sensitivity and referral characteristics to secondary care. Eur J Gastroenterol Hepatol. 2014;26(1):33-9.
110. Sapone A, Lammers KM, Casolaro V, Cammarota M, Giuliano MT, De Rosa M, et al. Divergence of gut permeability and mucosal immune gene expression in two gluten-associated conditions: celiac disease and gluten sensitivity. BMC Med. 2011;9:23.
111. Biesiekierski JR, Peters SL, Newnham ED, Rosella O, Muir JG, Gibson PR. No effects of gluten in patients with self-reported non-celiac gluten sensitivity after dietary reduction of fermentable, poorly absorbed, short-chain carbohydrates. Gastroenterology. 2013;145(2):320-8 e1-3.
112. Aziz I, Hadjivassiliou M, Sanders DS. The spectrum of noncoeliac gluten sensitivity. Nat Rev Gastroenterol Hepatol. 2015;12(9):516-26.
113. Catassi C, Kryszak D, Louis-Jacques O, Duerksen DR, Hill I, Crowe SE, et al. Detection of Celiac disease in primary care: a multicenter case-finding study in North America. Am J Gastroenterol. 2007;102(7):1454-60.

114. Chou R, Bougatsos C, Blazina I, Mackey K, Grusing S, Selph S. Screening for Celiac Disease: Evidence Report and Systematic Review for the US Preventive Services Task Force. JAMA. 2017;317(12):1258-68.
115. Jin J. Screening for Celiac Disease. JAMA. 2017;317(12):1286.
116. Force USPST, Bibbins-Domingo K, Grossman DC, Curry SJ, Barry MJ, Davidson KW, et al. Screening for Celiac Disease: US Preventive Services Task Force Recommendation Statement. JAMA. 2017;317(12):1252-7.
117. Choung RS, Murray JA. The US Preventive Services Task Force Recommendation on Screening for Asymptomatic Celiac Disease: A Dearth of Evidence. JAMA. 2017;317(12):1221-3.
118. Lau MS, Mooney PD, White WL, Appleby V, Moreea S, Haythem I, et al. Erratum to: 'Pre-endoscopy point of care test (Simtomax- IgA/IgG-Deamidated Gliadin Peptide) for coeliac disease in iron deficiency anaemia: diagnostic accuracy and a cost saving economic model'. BMC Gastroenterol. 2016;16(1):122.
119. Leffler D, Schuppan D, Pallav K, Najarian R, Goldsmith JD, Hansen J, et al. Kinetics of the histological, serological and symptomatic responses to gluten challenge in adults with coeliac disease. Gut. 2013;62(7):996-1004.
120. Raju SA, Mooney PD, Aziz I, Kurien M, Sanders DS. Letter: gluten challenge in the era of noncoeliac gluten sensitivity--a change in clinical practice? Aliment Pharmacol Ther. 2016;43(5):656.
121. Korponay-Szabó IR, Szabados K, Pusztai J, Uhrin K, Ludmány E, Nemes E, et al. Population screening for coeliac disease in primary care by district nurses using a rapid antibody test: diagnostic accuracy and feasibility study. BMJ. 2007;335(7632):1244-7.
122. Almazán MV, Ortega E, Moreno Torres R, Tovar M, Romero J, López-Casado M, et al. Diagnostic screening for subclinical celiac disease using a rapid test in children aged 2-4. Pediatr Res. 2015;78(3):280-5.
123. Nemec G, Ventura A, Stefano M, Di Leo G, Baldas V, Tommasini A, et al. Looking for celiac disease: diagnostic accuracy of two rapid commercial assays. Am J Gastroenterol. 2006;101(7):1597-600.
124. Laadhar L, Kallel-Sellami M, Zitouni M, Mehrezi A, Makni S, Ben Hariz M. Is the rapid whole blood test useful for diagnosis and monitoring celiac disease in children? Tunis Med. 2011;89(1):16-7.
125. Baviera LC, Aliaga ED, Ortigosa L, Litwin N, Peña-Quintana L, Méndez V, et al. Celiac disease screening by immunochromatographic visual assays: results of a multicenter study. J Pediatr Gastroenterol Nutr. 2007;45(5):546-50.
126. Lewis NR, Scott BB. Systematic review: the use of serology to exclude or diagnose coeliac disease (a comparison of the endomysial and tissue transglutaminase antibody tests). Aliment Pharmacol Ther. 2006;24(1):47-54.
127. Lewis NR, Scott BB. Meta-analysis: deamidated gliadin peptide antibody and tissue transglutaminase antibody compared as screening tests for coeliac disease. Aliment Pharmacol Ther. 2010;31(1):73-81.
128. Niveloni S, Sugai E, Cabanne A, Vazquez H, Argonz J, Smecuol E, et al. Antibodies against synthetic deamidated gliadin peptides as predictors of celiac disease: prospective assessment in an adult population with a high pretest probability of disease. Clin Chem. 2007;53(12):2186-92.
129. Rashtak S, Ettore MW, Homburger HA, Murray JA. Comparative usefulness of deamidated gliadin antibodies in the diagnosis of celiac disease. Clin Gastroenterol Hepatol. 2008;6(4):426-32; quiz 370.
130. Abrams JA, Brar P, Diamond B, Rotterdam H, Green PH. Utility in clinical practice of immunoglobulin a anti-tissue transglutaminase antibody for the diagnosis of celiac disease. Clin Gastroenterol Hepatol. 2006;4(6):726-30.

131. Beltran L, Koenig M, Egner W, Howard M, Butt A, Austin MR, et al. High-titre circulating tissue transglutaminase-2 antibodies predict small bowel villous atrophy, but decision cut-off limits must be locally validated. Clin Exp Immunol. 2014;176(2):190-8.
132. Venugopal G, Mechenro J, Makharia G, Singh A, Pugazhendhi S, Balamurugan R, et al. Sequential testing with different tissue transglutaminase antibodies, a new approach for diagnosis of celiac disease. Indian J Gastroenterol. 2017.
133. Kaukinen K, Turjanmaa K, Maki M, Partanen J, Venalainen R, Reunala T, et al. Intolerance to cereals is not specific for coeliac disease. Scand J Gastroenterol. 2000;35(9):942-6.
134. Coburn JA, Vande Voort JL, Lahr BD, Van Dyke CT, Kroning CM, Wu TT, et al. Human leukocyte antigen genetics and clinical features of self-treated patients on a gluten-free diet. J Clin Gastroenterol. 2013;47(10):828-33.
135. Kabbani TA, Vanga RR, Leffler DA, Villafuerte-Galvez J, Pallav K, Hansen J, et al. Celiac disease or non-celiac gluten sensitivity? An approach to clinical differential diagnosis. Am J Gastroenterol. 2014;109(5):741-6; quiz 7.
136. Sharma P, Scotland G, Cruickshank M, Tassie E, Fraser C, Burton C, et al. The clinical effectiveness and cost-effectiveness of point-of-care tests (CoaguChek system, INRatio2 PT/INR monitor and ProTime Microcoagulation system) for the self-monitoring of the coagulation status of people receiving long-term vitamin K antagonist therapy, compared with standard UK practice: systematic review and economic evaluation. Health Technol Assess. 2015;19(48):1-172.
137. Gliddon HD, Peeling RW, Kamb ML, Toskin I, Wi TE, Taylor MM. A systematic review and meta-analysis of studies evaluating the performance and operational characteristics of dual point-of-care tests for HIV and syphilis. Sex Transm Infect. 2017.
138. Hoerter NA, Shannahan SE, Suarez J, Lewis SK, Green PHR, Leffler DA, et al. Diagnostic Yield of Isolated Deamidated Gliadin Peptide Antibody Elevation for Celiac Disease. Dig Dis Sci. 2017;62(5):1272-6.
139. Leffler DA, Green PH, Fasano A. Extraintestinal manifestations of coeliac disease. Nat Rev Gastroenterol Hepatol. 2015;12(10):561-71.
140. Burger JP, Meijer JW, Wahab PJ. Routine duodenal biopsy to screen for coeliac disease is not effective. Neth J Med. 2013;71(6):308-12.
141. Halfdanarson TR, Litzow MR, Murray JA. Hematologic manifestations of celiac disease. Blood. 2007;109(2):412-21.
142. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. Public Health Nutr. 2009;12(4):444-54.
143. Guralnik JM, Eisenstaedt RS, Ferrucci L, Klein HG, Woodman RC. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. Blood. 2004;104(8):2263-8.
144. McIntyre AS, Long RG. Prospective survey of investigations in outpatients referred with iron deficiency anaemia. Gut. 1993;34(8):1102-7.
145. Corazza GR, Valentini RA, Andreani ML, D'Anchino M, Leva MT, Ginaldi L, et al. Subclinical coeliac disease is a frequent cause of iron-deficiency anaemia. Scand J Gastroenterol. 1995;30(2):153-6.
146. Unsworth DJ, Lock RJ, Harvey RF. Improving the diagnosis of coeliac disease in anaemic women. Br J Haematol. 2000;111(3):898-901.
147. Howard MR, Turnbull AJ, Morley P, Hollier P, Webb R, Clarke A. A prospective study of the prevalence of undiagnosed coeliac disease in laboratory defined iron and folate deficiency. J Clin Pathol. 2002;55(10):754-7.
148. Grisolano SW, Oxentenko AS, Murray JA, Burgart LJ, Dierkhising RA, Alexander JA. The usefulness of routine small bowel biopsies in evaluation of iron deficiency anemia. J Clin Gastroenterol. 2004;38(9):756-60.

149. Goddard AF, James MW, McIntyre AS, Scott BB, Gastroenterology BSo. Guidelines for the management of iron deficiency anaemia. Gut. 2011;60(10):1309-16.
150. Hopper AD, Cross SS, Hurlstone DP, McAlindon ME, Lobo AJ, Hadjivassiliou M, et al. Pre-endoscopy serological testing for coeliac disease: evaluation of a clinical decision tool. BMJ. 2007;334(7596):729.
151. Gonen. Diagnostic yield of routine duodenal biopsies in iron deficiency anaemia: a study from Western Anatolia. European Journal of Gastroenterology Hepatology. 2007;19(1):37-41.
152. Gulen H, Kasirga E, Yildirim SA, Kader S, Sahin G, Ayhan S. Diagnostic yield of upper gastrointestinal endoscopy in the evaluation of iron deficiency anemia in older children and adolescents. Pediatr Hematol Oncol. 2011;28(8):694-701.
153. Tischendorf JJ, Wopp K, Streetz KL, Bach J, Meyer M, Tischendorf S, et al. [The value of duodenal biopsy within routine upper endoscopy: a prospective study in 1000 patients]. Z Gastroenterol. 2008;46(8):771-5.
154. Dubin SM, Kwong W, Kalmaz D, Savides T. Low yield of routine duodenal biopsies for evaluation of abdominal pain. World Journal Of Gastroenterology. 2015;21(24):7495-9.
155. Bai JC, Mazure RM, Vazquez H, Niveloni SI, Smecuol E, Pedreira S, et al. Whipple's disease. Clin Gastroenterol Hepatol. 2004;2(10):849-60.
156. Patey-Mariaud de Serre N, Reijasse D, Verkarre V, Canioni D, Colomb V, Haddad E, et al. Chronic intestinal graft-versus-host disease: clinical, histological and immunohistochemical analysis of 17 children. Bone Marrow Transplant. 2002;29(3):223-30.
157. Luzi G, Zullo A, Iebba F, Rinaldi V, Sanchez Mete L, Muscaritoli M, et al. Duodenal pathology and clinical-immunological implications in common variable immunodeficiency patients. Am J Gastroenterol. 2003;98(1):118-21.
158. Khodadad A, Aghamohammadi A, Parvaneh N, Rezaei N, Mahjoob F, Bashashati M, et al. Gastrointestinal manifestations in patients with common variable immunodeficiency. Dig Dis Sci. 2007;52(11):2977-83.
159. Masia R, Peyton S, Lauwers GY, Brown I. Gastrointestinal biopsy findings of autoimmune enteropathy: a review of 25 cases. Am J Surg Pathol. 2014;38(10):1319-29.
160. Marthey L, Cadiot G, Seksik P, Pouderoux P, Lacroute J, Skinazi F, et al. Olmesartan-associated enteropathy: results of a national survey. Aliment Pharmacol Ther. 2014;40(9):1103-9.
161. Silvester JA, Weiten D, Graff LA, Walker JR, Duerksen DR. Living gluten-free: adherence, knowledge, lifestyle adaptations and feelings towards a gluten-free diet. J Hum Nutr Diet. 2016;29(3):374-82.
162. Hall NJ, Rubin GP, Charnock A. Intentional and inadvertent non-adherence in adult coeliac disease. A cross-sectional survey. Appetite. 2013;68:56-62.
163. Leffler DA, Dennis M, Hyett B, Kelly E, Schuppan D, Kelly CP. Etiologies and predictors of diagnosis in nonresponsive celiac disease. Clin Gastroenterol Hepatol. 2007;5(4):445-50.
164. Gray AM, Papanicolas IN. Impact of symptoms on quality of life before and after diagnosis of coeliac disease: results from a UK population survey. BMC Health Serv Res. 2010;10:105.
165. Sainsbury A, Sanders DS, Ford AC. Prevalence of irritable bowel syndrome-type symptoms in patients with celiac disease: a meta-analysis. Clin Gastroenterol Hepatol. 2013;11(4):359-65 e1.
166. Rubio-Tapia A, Rahim MW, See JA, Lahr BD, Wu TT, Murray JA. Mucosal recovery and mortality in adults with celiac disease after treatment with a gluten-free diet. Am J Gastroenterol. 2010;105(6):1412-20.
167. Sharkey LM, Corbett G, Currie E, Lee J, Sweeney N, Woodward JM. Optimising delivery of care in coeliac disease - comparison of the benefits of repeat biopsy and serological follow-up. Aliment Pharmacol Ther. 2013;38(10):1278-91.

168. Wahab PJ, Meijer JW, Mulder CJ. Histologic follow-up of people with celiac disease on a gluten-free diet: slow and incomplete recovery. Am J Clin Pathol. 2002;118(3):459-63.
169. Lebwohl B, Michaelsson K, Green PH, Ludvigsson JF. Persistent mucosal damage and risk of fracture in celiac disease. J Clin Endocrinol Metab. 2014;99(2):609-16.
170. Kavic SM, Basson MD. Complications of endoscopy. Am J Surg. 2001;181(4):319-32.
171. Committee ASoP, Ben-Menachem T, Decker GA, Early DS, Evans J, Fanelli RD, et al. Adverse events of upper GI endoscopy. Gastrointest Endosc. 2012;76(4):707-18.
172. Bai JC, Fried M, Corazza GR, Schuppan D, Farthing M, Catassi C, et al. World Gastroenterology Organisation global guidelines on celiac disease. J Clin Gastroenterol. 2013;47(2):121-6.
173. Rostom A, Murray JA, Kagnoff MF. American Gastroenterological Association (AGA) Institute technical review on the diagnosis and management of celiac disease. Gastroenterology. 2006;131(6):1981-2002.
174. Marlou PMA, Daniel AL, Ciaran PK, Detlef S, Robert MN, Jeffrey DG, et al. Serum I- FABP Detects Gluten Responsiveness in Adult Celiac Disease Patients on a Short-Term Gluten Challenge. The American Journal of Gastroenterology. 2016.
175. Moreno ML, Cebolla A, Munoz-Suano A, Carrillo-Carrion C, Comino I, Pizarro A, et al. Detection of gluten immunogenic peptides in the urine of patients with coeliac disease reveals transgressions in the gluten-free diet and incomplete mucosal healing. Gut. 2017;66(2):250-7.
176. Adriaanse M, Leffler DA. Serum markers in the clinical management of celiac disease. Dig Dis. 2015;33(2):236-43.
177. Pyle GG, Paaso B, Anderson BE, Allen D, Marti T, Khosla C, et al. Low-dose gluten challenge in celiac sprue: malabsorptive and antibody responses. Clin Gastroenterol Hepatol. 2005;3(7):679-86.
178. Vilela EG, de Abreu Ferrari Mde L, de Gama Torres HO, Martins FP, Goulart EM, Lima AS, et al. Intestinal permeability and antigliadin antibody test for monitoring adult patients with celiac disease. Dig Dis Sci. 2007;52(5):1304-9.
179. Moron B, Verma AK, Das P, Taavela J, Dafik L, Diraimondo TR, et al. CYP3A4-catalyzed simvastatin metabolism as a non-invasive marker of small intestinal health in celiac disease. Am J Gastroenterol. 2013;108(8):1344-51.
180. Kaukinen K, Sulkanen S, Maki M, Collin P. IgA-class transglutaminase antibodies in evaluating the efficacy of gluten-free diet in coeliac disease. Eur J Gastroenterol Hepatol. 2002;14(3):311-5.
181. Vahedi K, Mascart F, Mary JY, Laberenne JE, Bouhnik Y, Morin MC, et al. Reliability of antitransglutaminase antibodies as predictors of gluten-free diet compliance in adult celiac disease. Am J Gastroenterol. 2003;98(5):1079-87.
182. Leffler DA, Edwards George JB, Dennis M, Cook EF, Schuppan D, Kelly CP. A prospective comparative study of five measures of gluten-free diet adherence in adults with coeliac disease. Aliment Pharmacol Ther. 2007;26(9):1227-35.
183. Biagi F, Bianchi PI, Marchese A, Trotta L, Vattiato C, Balduzzi D, et al. A score that verifies adherence to a gluten-free diet: a cross-sectional, multicentre validation in real clinical life. Br J Nutr. 2012;108(10):1884-8.
184. George DA, Hui LL, Rattehalli D, Lovatt T, Perry I, Green M, et al. The role of near- patient coeliac serology testing in the follow- up of patients with coeliac disease. Frontline Gastroenterol. 2014;5(1):20.
185. Volta U, Granito A, Fiorini E, Parisi C, Piscaglia M, Pappas G, et al. Usefulness of antibodies to deamidated gliadin peptides in celiac disease diagnosis and follow-up. Dig Dis Sci. 2008;53(6):1582-8.
186. Spatola BN, Kaukinen K, Collin P, Maki M, Kagnoff MF, Daugherty PS. Persistence of elevated deamidated gliadin peptide antibodies on a gluten-free diet indicates nonresponsive coeliac disease. Aliment Pharmacol Ther. 2014;39(4):407-17.

187. Monzani A, Rapa A, Fonio P, Tognato E, Panigati L, Oderda G. Use of deamidated gliadin peptide antibodies to monitor diet compliance in childhood celiac disease. J Pediatr Gastroenterol Nutr. 2011;53(1):55-60.
188. de Chaisemartin L, Meatchi T, Malamut G, Fernani-Oukil F, Hosking F, Rault D, et al. Application of Deamidated Gliadin Antibodies in the Follow-Up of Treated Celiac Disease. PLoS One. 2015;10(8):e0136745.
189. Bannister EG, Cameron DJ, Ng J, Chow CW, Oliver MR, Alex G, et al. Can celiac serology alone be used as a marker of duodenal mucosal recovery in children with celiac disease on a gluten-free diet? Am J Gastroenterol. 2014;109(9):1478-83.
190. Kaukinen K, Peraaho M, Lindfors K, Partanen J, Woolley N, Pikkarainen P, et al. Persistent small bowel mucosal villous atrophy without symptoms in coeliac disease. Aliment Pharmacol Ther. 2007;25(10):1237-45.
191. Bardella MT, Velio P, Cesana BM, Prampolini L, Casella G, Di Bella C, et al. Coeliac disease: a histological follow-up study. Histopathology. 2007;50(4):465-71.
192. Lanzini A, Lanzarotto F, Villanacci V, Mora A, Bertolazzi S, Turini D, et al. Complete recovery of intestinal mucosa occurs very rarely in adult coeliac patients despite adherence to gluten-free diet. Aliment Pharmacol Ther. 2009;29(12):1299-308.
193. Kotze LM, Brambila Rodrigues AP, Kotze LR, Nisihara RM. A Brazilian experience of the self transglutaminase-based test for celiac disease case finding and diet monitoring. World J Gastroenterol. 2009;15(35):4423-8.
194. Crovella S, Brandao L, Guimaraes R, Filho JL, Arraes LC, Ventura A, et al. Speeding up coeliac disease diagnosis in the developing countries. Dig Liver Dis. 2007;39(10):900-2.
195. Schyum AC, Rumessen JJ. Serological testing for celiac disease in adults. United European Gastroenterol J. 2013;1(5):319-25.
196. Christensen TD, Larsen TB. Precision and accuracy of point-of-care testing coagulometers used for self-testing and self-management of oral anticoagulation therapy. J Thromb Haemost. 2012;10(2):251-60.
197. Vallejo-Diez S, Bernardo D, Moreno Mde L, Munoz-Suano A, Fernandez-Salazar L, Calvo C, et al. Detection of specific IgA antibodies against a novel deamidated 8-Mer gliadin peptide in blood plasma samples from celiac patients. PLoS One. 2013;8(11):e80982.
198. Pichler J, Zilbauer M, Torrente F, Heuschkel R, Phillips A, Salvestrini C. Feasibility of a finger prick-based self-testing kit in first- and second-degree relatives of children with coeliac disease. World J Gastroenterol. 2011;17(14):1840-3.
199. Oliveira A, Trindade E, Tavares M, Lima R, Terra M, Dias JA. Celiac disease in first degree relatives of celiac children. Arq Gastroenterol. 2012;49(3):204-7.
200. Polanco I, Koester Weber T, Martinez-Ojinaga E, Molina M, Sarria J. Efficacy of a point-of-care test based on deamidated gliadin peptides for the detection of celiac disease in pediatric patients. Rev Esp Enferm Dig. 2017;109(11):743-8.
201. Karagiozoglou-Lampoudi T, Zellos A, Vlahavas G, Kafritsa Y, Roma E, Papadopoulou A, et al. Screening for coeliac disease in preschool Greek children: the feasibility study of a community-based project. Acta Paediatr. 2013;102(7):749-54.
202. Popp A, Jinga M, Jurcut C, Balaban V, Bardas C, Laurila K, et al. Fingertip rapid point-of-care test in adult case-finding in coeliac disease. BMC Gastroenterol. 2013;13:115.
203. Costa S, Astarita L, Ben-Hariz M, Currò G, Dolinsek J, Kansu A, et al. A point-of-care test for facing the burden of undiagnosed celiac disease in the Mediterranean area: a pragmatic design study. BMC Gastroenterol. 2014;14:219.
204. Ceylan n, Demiroren K. The prevalence of celiac disease in healthy school children in Van City, east of Turkey: a screening study using a rapid test Medical Science and Discovery. 2016;3(3):130-3.

205. Ferre-López S, Ribes-Koninckx C, Genzor C, Gamen S, Peña L, Ortigosa L, et al. Immunochromatographic sticks for tissue transglutaminase and antigliadin antibody screening in celiac disease. Clin Gastroenterol Hepatol. 2004;2(6):480-4.

Appendix 1: A Supplementary Table of All the Studies on the Commercially Available point of care tests.

Test	Reference	Country	n	Population	CD prevalence	Sensitivity	Specificity	Study Limitations
Biocard	(123) Nemec 2006	Italy	151	Adult & Paediatric	33.8%	90.2%	100%	Only positive POCT/serology biopsied. High pre-test probability.
Biocard	(82) Raivio 2006	Hungary and Finland	150	Paediatric	30%	95.5%	97.1%	Only positive POCT/serology biopsied. Sensitivities compared against serology. High pre-test probability.
Biocard	(121) Korponay-Szabo 2007	Finland	43	Adults	43%	91.7%	78.9%	High pre-test probability.
Biocard	(121) Korponay-Szabo 2007	Hungary	2676	Paediatric	1.2%	78.1%	100%	Only positive POCT/serology biopsied.
Biocard	(194) Crovella 2007	Brazil	1074	Adult	0.84%	No data	100% in urban, 76% in suburban	Only positive POCT/serology biopsied.
Biocard	(83) Raivio 2008	Finland	242	Paediatric	57.4%	93%	94%	High pre-test probability.
Biocard	(193) Kotze 2009	Brazil	300	Adult & Paediatric	4.7%	No data	No data	Only positive POCT or high suspicion of CD biopsied.
Biocard	(124) Laadhar 2011	Tunisia	57	Paediatric	no data	100%	96.4%	Sensitivities compared against TTG.
Biocard	(198) Pichler 2011	UK	196	Adult & Paediatric	1.5%	No data	No data	Only positive POCT biopsied.
Biocard	(16) Alarida 2011	Libya	2920	Paediatric	0.79%	58%	40%	Only positive POCT/serology biopsied.
Biocard	(199) Oliviera 2012	Portugal	268	Adult	2.6%	No data	No data	Only positive POCT biopsied.
Biocard	(200) Hariz 2011	Tunisia	2064	Paediatric	0.24-0.34%	100%	100%	Only positive POCT/serology biopsied.
Biocard	(201) Karagiozoglu-Lampoudi 2013	Greece	1136	Paediatric	0.65%	No data	No data	Only those with positive POCT went on to have EMA and TTG. Only positive serology biopsied.
Biocard	(202) Popp 2013	Romania	148	Adult & Paediatric	6%	92.3%	100%	Only positive POCT/serology biopsied.

Biocard	(84) Mooney	UK	576	Adult	22.4%	70.1%	96.6%	High pre-test probability.
Biocard	(203) Costa 2014	Italy	206	Adult	44.%	90%	98.7%	Only positive POCT/serology biopsied. High pre-test probability.
Biocard	(203) Costa 2014	Italy	85	Paediatric	11.80%	97.1%	94.1%	Only positive POCT/serology biopsied. Slightly high pre-test probability.
Biocard	(203) Costa 2014	Italy	3559	Paediatric	0.50%	No data	No data	Only positive POCT/serology biopsied
Biocard	(203) Costa 2014	Slovenia	1480	Paediatric	0.47%	No data	No data	Only positive POCT/serology biopsied
Biocard	(203) Costa 2014	Turkey	785	Paediatric	0.13%	No data	No data	Only positive POCT/serology biopsied
Biocard	(85) Singh 2014	India	124	Paediatric	64.70%	83.6%	90%	High pre-test probability.
Biocard	(87) Mooney 2015	UK	55	Adults	65.40%	72.2%	No data	Sensitivities compared against EMA. High pre-test probability.
Biocard	(204) Ceylan 2016	Turkey	1003	Paediatric	0.3%	No data	No data	None of the patients had biopsies. Prevalence based on positive POCT.
Stick CD1	(205) Ferre-Lopez 2004	Spain	286	Paediatric	53.5%	97.1%	99%	High pre-test probability.
Stick CD2	(205) Ferre-Lopez 2004	Spain	49	Adult	61.2%	83.3%	100%	High pre-test probability.
Stick CD1	(123) Nemec 2006	Italy	329	Adult & Paediatric	34.7%	100%	94.9%	Only positive POCT/serology biopsied. Sensitivities compared against TTG. High pre-test probability.
Stick CD1	(125) Baviera 2007	Spain and Latin America	185	Paediatric	61.1%	96.5%	98.6%	High pre-test probability.
Stick CD2	(125) Baviera 2007	Spain and Latin America	185	Paediatric	61.1%	94.5%	98.6%	High pre-test probability.
Stick CD1	(194) Crovella 2007	Brazil	1074	Adult	0.84%	No data	100% in urban, 76% in suburban	Only positive POCT biopsied.
Simple CD1WB	(122) Almazan 2015	Spain	198	Paediatric	3%	16.6%	98.9%	Only positive POCT/serology biopsied.
Simple CD2WB	(122) Almazan 2015	Spain	198	Paediatric	3%	100%	89.1%	Only positive POCT/serology biopsied.

Celiac Quick Test	(184) George 2014	UK	80	Adults with known CD*	100%	Predicting remission 93.5% (serum)	Predicting remission 94.9% (serum)	Only symptomatic, non-compliant or positive serology biopsied. Sensitivities compared against serology.
Celiac Quick Test	(184) George 2014	UK	80	Adults with known CD*	100%	Predicting remission 77.8% (whole blood)	Predicting remission 100% (whole blood)	Only symptomatic, non-compliant or positive serology biopsied. Sensitivities of POCT compared against serology.
Celiac Quick Test	(87) Mooney 2015	UK	55	Adults	65.40%	77.8%	no data	Sensitivities compared against EMA. High pre-test probability.
Simtomax	(90) Bienvenu 2012	France	250	Paediatric	9.6%	95%	93.1%	Only positive TTG biopsied. Sensitivities compared against serology. High risk/CD symptoms
Simtomax	(89) Benkebil 2013	Switzerland	66	Adult & Paediatric	12%	100.%	93.1%	Only positive POCT/serology biopsied. Slightly high pre-test probability. FDR and CD symptoms
Simtomax	(89) Benkebil 2013	Switzerland	46	Adult & Paediatric	100%	Predicting remission 78.9%	Predicting remission 95.7%	Only positive POCT/serology biopsied. Sensitivities compared against serology.
Simtomax	(88) Bienvenu 2014	France	45	Paediatric	17.8%	100.%	89.2%	Only positive POCT/serology biopsied. High pre-test probability. Retrospective Simtomax test on IgA def, some missing data as retrospective
Simtomax	(87) Mooney 2015	UK	55	Adults	65.40%	94.4%	no data	High pre-test probability.
Simtomax	(87) Mooney 2015	UK	508	Adults	13.40%	92.7%	85.2%	Slightly high pre-test probability.
Simtomax	(200) Polanco 2017	Spain	100	Paediatric	48%	95.8%	98.1%	High prevalence. Reference standard for POCT based on new ESPGHAN guidelines.

Appendix 2: Participant questionnaire (Chapter 3)

Participant questionnaire

1. Are you under 18 years of age?
2. Have you been diagnosed with coeliac disease?
3. Have you previously been or are you currently being investigated for coeliac disease?

If your answers are no to all 3 questions above, please continue to question 4 and 5.

If any of your above answers is yes, you are not eligible to take part in the study.

4. Do you suffer from any of the following?

- Persistent unexplained gastrointestinal symptoms (bloating, abdominal pain or discomfort, diarrhoea, constipation, urgency, nausea/vomiting, indigestion)
- Prolonged fatigue
- Unexpected weight loss
- Severe or persistent mouth ulcers
- Unexplained iron, vitamin B12 or folate deficiency
- Type 1 diabetes
- Autoimmune thyroid disease
- Irritable bowel syndrome
- Osteoporosis or osteomalacia
- Unexplained neurological symptoms (particularly numbness and tingling in your extremeties, or problems with your balance)
- Unexplained fertility problems or recurrent miscarriage
- Persistently abnormal liver blood tests
- Dental enamel defects (discoloured, grooves and pits or yellow/brown spots on teeth surfaces; irregular shaped teeth)

5. Do you have any first degree relatives with coeliac disease?

If any of your answers is yes to question 4 or 5, you will be eligible to take part in the study.

Demographic details:
Name: Gender: DOB:
Contact number:
Address

Appendix 3: Study recruitment poster for pharmacy customers (Chapter 3)

Appendix 4: Patient information sheet (Chapter 3)

Participant information sheet

Study title: Case Finding for Coeliac Disease using a Point of Care Test in a Pharmacy Setting: A Feasibility Study

Invitation: We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of the team will go through the information sheet with you and answer any questions you have. This should take about five to ten minutes. Talk to others about the study if you wish. Ask us if there is anything that is not clear.

What is the purpose of the study?
Coeliac disease is an autoimmune condition in which the body attacks the lining of the small bowel. The immune reaction is triggered by gluten, a protein found in wheat, barley and rye.
Coeliac disease affects approximately 1 in 100 people and is treated with a gluten-free diet.
However, coeliac disease is underdiagnosed- for every patient known to have coeliac disease, 5 patients remain undiagnosed. Undiagnosed individuals could suffer from long term health complications and a reduced quality of life.
A new rapid antibody test kit for coeliac disease has become available, producing coeliac antibody results within 10 minutes. This study aims to assess whether using this test kit in pharmacies would help to increase the detection of coeliac disease. Those with positive test results will be advised to visit their GP to be referred for a gastroscopy to confirm the diagnosis.

Why have I been invited?
You have been invited because you may have risk factors for or symptoms due to coeliac disease.

Do I have to take part?
No. It is up to you whether or not you decide to take part in this study. If you do decide to take part, you will be given this information sheet to keep and asked to sign a consent form. If you choose not to take part, you do not need to give a reason and your care will not be affected in any way.

What will happen to me if I take part?
You will have a finger prick test at the pharmacy to assess if you potentially have coeliac disease. The test results will be available within 10 minutes.

If the test result is positive, you will be advised to visit your GP to be referred for a gastroscopy (camera test) at the Royal Hallamshire Hospital. This is to obtain biopsies from your small bowel to confirm the diagnosis of coeliac disease as per usual practice. The chance of being diagnosed with coeliac disease with a positive finger prick test is about 50% after further investigation with

a gastroscopy. The accuracy of this finger prick test is high, and is comparable to the conventional blood tests we currently use to detect coeliac disease.

A gastroscopy is a procedure where a thin, flexible tube called an endoscope is used to look inside the oesophagus, stomach and first part of the small intestine. A gastroscopy often takes less than 10 minutes. Before the procedure, your throat will be numbed with a local anaesthetic spray. You can also choose to have a sedative, if you prefer. The endoscope will enter the back of your mouth and it will then be guided down your oesophagus into your stomach. Tiny samples will be obtained from your small intestine. A diagnostic gastroscopy is a very safe procedure. Possible complications that can occur include: a reaction to the sedative, which can cause problems with your breathing, heart rate and blood pressure, internal bleeding (usually minor and self-limiting if it occurs), and very rarely, tearing of the gut lining (perforation), occurring in less than 1 in 3,000 cases.

If the test result is negative, you most probably do not have coeliac disease. You do not need to do anything and a gastroscopy is not required for a negative finger prick test. If your symptoms persist, please consult your GP.

When do I have to decide?
It is up to you. You can decide now, or think about it and let us know at a later date as long as the study is running.

Will this affect my existing treatment?
No. This study does not involve administration of medications.

What are the possible disadvantages and risks of taking part?
The finger pin-prick test is minimally uncomfortable, and will take 10 minutes to obtain the results.

What are the possible benefits of taking part?
For those who are diagnosed with coeliac disease, you will benefit from an early diagnosis, meaning that you will receive treatment early, preventing long term complications and have an improved quality of life.

For those in whom coeliac disease has been ruled out, you may not have a direct benefit from the study, but your contribution will help us provide the best care for patients in the future.

How long will the study last?
The study will last approximately 18 months.

Will my taking part in the study be kept confidential?
Yes, your data will be kept confidential. It will be governed by the Data Protection Act (1998) and has Research Ethics Committee approval. Information will be completely anonymised if it is to be analysed or published outside the research team. Only the research team will be able to see

your personal information. The information that can identify you personally will NEVER be given to anyone else or published. Only relevant sections of your medical notes will be looked at by the research team, from regulatory authorities or from your NHS Trust. Personal data will only be stored on NHS password protected computers and when this is pseudonymised the identification list will be kept separate located on the NHS system.

What will happen if I don't want to carry on with the study?
You can withdraw your consent at any time in the future without giving a reason. If you withdraw your consent or become unable to continue to give informed consent, any information collected with consent will remain and be used in the study. No further information however will be collected and a record will be kept that you withdrew consent or were unable to continue to provide consent. Your care will not be affected in any way if you change your mind and withdraw from the study.

What if there is a problem?
Any complaints about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. Detailed information on who to contact are given at the end of this information sheet.

What will happen to the results of the research study?
We intend to publish the results of the research in peer reviewed journals, and to present them at scientific meetings. Professor Sanders holds an honorary post as the medical advisor to Coeliac UK and regularly speaks at their meetings. No identifiable data will be published.

Will my general practitioner (GP) be contacted?
Your GP will be informed if you have a positive finger prick test, in order to take it forward by referring you for a gastroscopy to confirm the diagnosis of coeliac disease.

Who is organising and funding the research?
The study is funded by Professor Sanders' research funds. Tillotts Pharma is providing the point of care test kits.

Who has reviewed the study?
All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by the Research Ethics Committee and the Sheffield Teaching Hospitals NHS Foundation Trust R&D Department (Clinical Research Office).

Thank you for taking the time to read this information sheet and for considering taking part in this study.

Further information and contact details:
If you want further general or specific information about the research, advice as to whether to participate or who to approach if you are unhappy with the study or want to take part or withdraw consent please contact:

Dr. Michelle Lau
Clinical Research Fellow in Gastroenterology
Michelle.lau3@sth.nhs.uk

If you have any complaints that you would like to be dealt with independently, please contact:

The Patient Services Team
Email: pst@sth.nhs.uk
Telephone: 0114 2712400

Or write to: The Medical Director
8 Beech Hill Rd
Sheffield
S10 2SB

Letter to the General Practitioner

Date:_____

Dear colleague,

Professor David Sanders' research team is currently running a case finding study for adult coeliac disease using a Point of Care Test in pharmacies across Sheffield. Participants with risk factors for or symptoms suggestive of coeliac disease are offered a Point of Care Test at pharmacies.

The Point of Care Test, Simtomax, detects coeliac disease associated IgA and IgG antibodies against a unique combination of deamidated gliadin peptides (DGP) as well as the total level of IgA. This ensures results are not influenced by patients with IgA deficiency, which is more common in people with coeliac disease than the general population. Preliminary data shows excellent and comparable sensitivities of Simtomax to conventional serology TTG and EMA.

Participant _____ has been tested positive. We would be grateful if you could refer him/her directly to Professor Sanders for a gastroscopy by the research team at the Royal Hallamshire Hospital, to obtain duodenal biopsies for histological confirmation of coeliac disease.

If you have any queries or would like further information about the research, please do not hesitate to contact:

Dr. Michelle Lau
Clinical Research Fellow in Gastroenterology
Michelle.lau3@sth.nhs.uk

Yours faithfully,

Dr. Michelle Lau
Clinical Research Fellow in Gastroenterology
Royal Hallamshire Hospital

Appendix 5: Patient consent form (Chapter 3)

Study Number: STH 19172

Participant Identification Number for this trial:

CONSENT FORM

Title of Project: Can a point of care test increase the detection of coeliac disease in a pharmacy setting?

Name of Researcher: Professor David Sanders, Dr. Michelle Lau

Please initial box

1. I confirm that I have read the information sheet dated 9/11/15 (version 2.0) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I consent to provide a blood sample for this study. I understand that my blood sample will be discarded after the test result is obtained. ☐
5. I understand that the information collected about me will be used to support other research in the future, and may be shared anonymously with other researchers. ☐
6. I agree to my General Practitioner being informed of my participation in the study. ☐
7. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Person taking consent	Date	Signature

Appendix 6: Acceptability questionnaires for point of care test and venepuncture (Chapter 4)

Acceptability questionnaire for patients in the intervention arm:	**Strongly disagree**	**Disagree**	**Neither**	**Agree**	**Strongly agree**
1. Blood collection process:					
a. The finger prick was not painful.					
b. The finger prick was quick and easy.					
2. Convenience:					
a. I am satisfied that I can have the test done within the same consultation which saves me from having to make a separate trip to the GP surgery or hospital to have my blood taken.					
b. I find it helpful to know the results within 10 minutes so it saves me from having to travel for a second consultation to obtain the results.					
3. Quality of care:					
a. I am satisfied that the test results are available immediately rather than having to wait for a few days or weeks.					
b. I find it useful to be able to discuss the results with the doctor within the same consultation.					
4. Preference:					
I prefer a finger prick test to a standard blood test taken from a vein.					

Acceptability questionnaire for venepuncture	Strongly disagree	Disagree	Neither	Agree	Strongly agree
1. Blood collection process:					
a. The blood test was not painful.					
b. The blood test was quick and easy.					
2. Convenience:					
a. I don't mind having to wait in line to have my blood taken in the phlebotomy department after seeing the doctor.					
b. I don't mind having to travel for a second consultation to obtain the results.					
3. Quality of care:					
a. I am satisfied that the test results are available within a few days to a few weeks.					
b. I don't mind having to wait for the next consultation to discuss the results with the doctor.					
4. Preference:					
I prefer a standard blood test taken from a vein to a finger prick test.					

Appendix 7: Patient information sheet (Chapters 4-6)

Patient Information Sheet

Study: Pre-endoscopy serological testing for coeliac disease; a novel approach using rapid antibody testing.

Investigators: Professor David Sanders, Dr. Michelle Lau, William White

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you
Ask us if you there is anything that is not clear or if you would like more information. Take time to decide whether you would like to participate or not. If you do decide to participate you will be given an information sheet and a copy of the signed consent form to keep.

What is the purpose of the study?
Coeliac disease is an autoimmune condition in which the body attacks the lining of the small bowel. The immune reaction is triggered by gluten, a protein found in wheat, barley and rye. Coeliac disease affects approximately 1% of the population and is treated with a gluten-free diet. It is diagnosed by taking a biopsy of the small bowel at endoscopy. It is important that people with a high risk for coeliac disease have biopsies taken. Currently routine laboratory blood tests are used to identify people at risk of coeliac disease. However, the results are not always available at the time of gastroscopy. A new test has become available that uses a pinprick of blood from the finger. A result is available within ten minutes. This test could potentially be used in the endoscopy department to identify those people requiring biopsy. This study aims to assess the accuracy of this test in the users of the endoscopy department to see if it could provide a faster, more convenient alternative to the traditional blood test.

Why have I been invited?
Your doctor has requested that you have a gastroscopy with biopsies and standard blood tests for coeliac disease as part of your routine medical care.

Do I have to take part?
It is up to you to decide. We would ask you to sign a consent form to show that you have agreed to take part. You are free to withdraw at any time without giving a reason. This would not affect the standard of care you receive.

What will happen to me if I take part?
At one of your routine appointments either in the outpatient clinic or in endoscopy, you will have a blood test. This is the test under evaluation and the results produced have not yet been validated (that is the purpose of this study). For this reason, you will not be told the result. The result of your test has no bearing on the care that you will receive.

What are the possible disadvantages and risks of taking part?
The blood test is minimally uncomfortable.

What are the possible benefits of taking part?
As an individual you will not benefit directly from the study but your contribution will help us provide the best care for patients in the future.

What if there is a problem?
If you have concerns about any aspect of the study you should ask to speak to Dr Michelle Lau, clinical research fellow on 01142261179. She will do her best to answer any questions. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.

Will my taking part in this study be kept confidential?
Identifiable details will not form any part of the database. Manual files are stored in a locked room with access only available to researchers. Files on NHS computers will require individual log on and password identification in keeping with standard Trust confidentiality practices. Databases will only be accessible by the research team who will also have have access to participants' personal data. Consent would be sought should monitors or auditors from the NHS require access. In the final analysis no identifiable data will be used. The database will be kept for 5 years after which time in would be destroyed in line with current practice.

What will happen to any samples I give?
The pin prick test is a ‘once only’ test and generates no storable sample.

What will happen to the results of the research study?
It is intended to publish the results of the research in peer reviewed journals, and to present them at scientific meetings. Professor Sanders holds an honorary post as medical advisor to Coeliac UK and regularly speaks at their meetings. No identifiable data will be published.

Who is organising and funding the research?
The study is sponsored by Professor Sanders’ research funds. Tillotts is providing the testing kits.

Who has reviewed the study?
All research in the NHS is looked at by an independent group called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by Hull and East Riding Research Ethics Committee.

Further information and contact details
If you have any questions about this study please contact Dr Michelle Lau, Clinical Research Fellow on 0114 2261179 or michelle.lau3@sth.nhs.uk

Appendix 8: Patient consent form (Chapters 4-6)

Consent Form

Title of project: Pre-endoscopy serological testing for coeliac disease; a novel approach using rapid antibody testing.

Investigators: Professor David Sanders, Dr. Michelle Lau, William White

Patient ID number:

Please initial each box with respect to the following:

☐ I confirm that I have read and understand the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

☐ I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

☐ I understand that relevant sections of data collected during the study may be looked at by responsible individuals from Sheffield Teaching Hospitals NHS Trust, or from regulatory authorities where it is relevant to my taking part in this research. I give permission for these individuals to have access to the data I have contributed.

☐ I agree to take part in the above study.

1 copy for patient and 1 for researcher file

_____

Name of patient Signature Date

_____

Name of person taking consent Signature Date

_____

Researcher Signature Date

Appendix 9: Health dietary adherence questionnaire for patients with known coeliac disease on a gluten free diet (Chapter 6)

Date _____
Name _____
Date of Birth _____

How long have you been on a gluten free diet?

_____

Race/Ethnicity:
White/Caucasian ☐ Afro-Caribbean ☐ Asian ☐ Other …… ☐

1. Compared to people of your own age, how would you rate your overall health?
Excellent ☐ Good ☐ Fair ☐ Poor ☐ Very poor ☐

2. Are you on a Gluten Free Diet Yes / No If yes :
Self-imposed ☐ Dietician advised ☐

3. How would you describe your bowel habit?
Regular/Normal ☐ Constipated ☐ Diarrhoea/Looseness ☐

Alternating constipation diarrhoea ☐

4. On average how often do you open your bowels?
Twice per week or less ☐ Alternate days ☐

Once per day ☐ Twice per day ☐ More than twice per day ☐

5. Do you currently have any of these symptoms?
Flatulence ☐ Bloating ☐ Floating stools ☐

Mucus in stools ☐ Urgency ☐

Nocturnal diarrhoea ☐ Weight loss ☐

6. Any other symptoms?
……

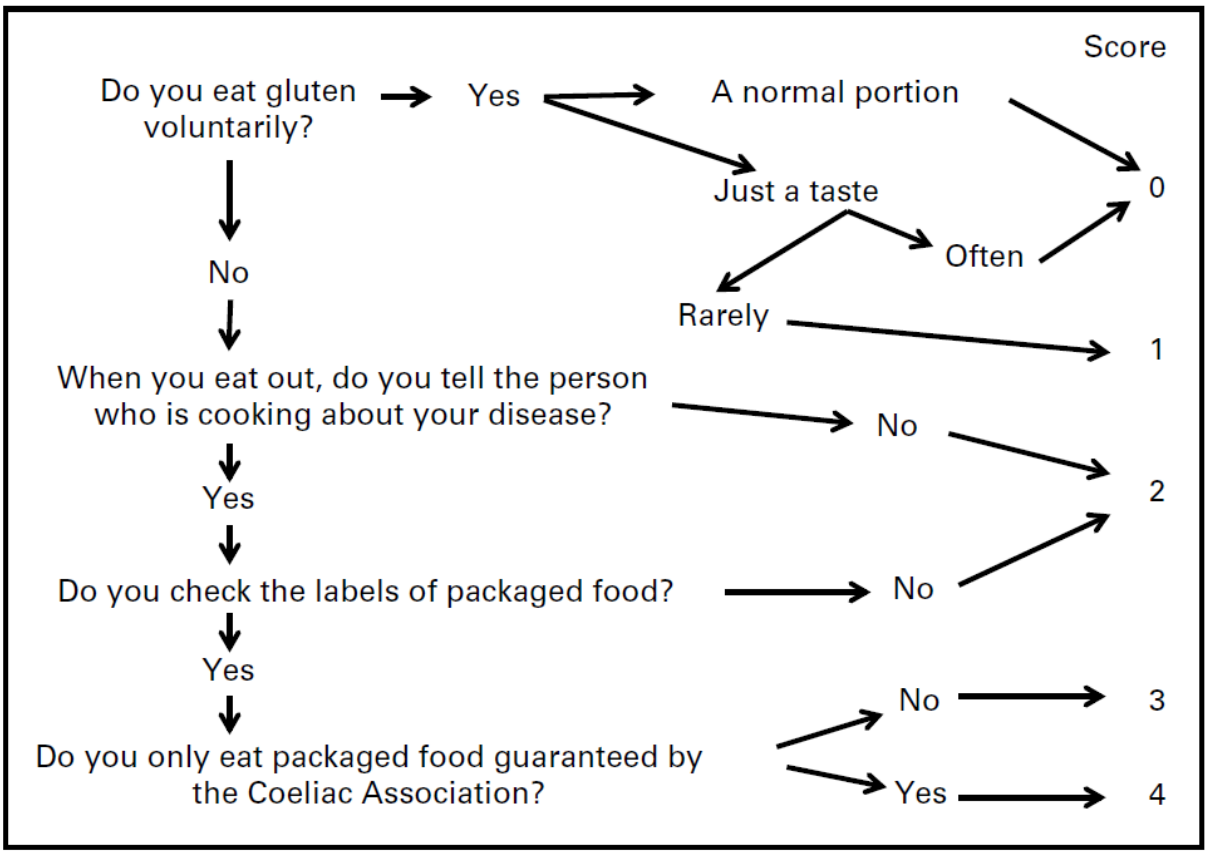
7. With regard to your energy level, do you feel:
Less tired than the people around you ☐
Similar energy to the people around you ☐
More tired than the people around you ☐
Tired all the time ☐

Appendix 10: Biagi dietary adherence questionnaire for patients with known coeliac disease on a gluten free diet (Chapter 6)

A score that verifies adherence to a gluten-free diet: a cross-sectional, multicentre validation in real clinical life

Federico Biagi, Paola Ilaria Bianchi, Alessandra Marchese, Lucia Trotta, Claudia Vattiato, Davide Balduzzi, Giovanna Brusco, Alida Andrealli, Fabio Cisarò, Marco Astegiano, Salvatore Pellegrino, Giuseppe Magazzù, Catherine Klersy and Gino Roberto Corazza

British Journal of Nutrition / *FirstView* Article / January 2006, pp 1 - 5
DOI: 10.1017/S0007114511007367, Published online: 10 February 2012



Publications derived from this body of work

Original research articles:

1. Chapter 3: Coeliac disease detection with an IgA/IgG-deamidated gliadin peptide based point of care test in community pharmacies.

   Lau MS, Sanders DS.

   Int J Clin Pharm. 2019 (in press)

2. Chapter 4: Office-Based Point of Care Testing (IgA/IgG-Deamidated Gliadin Peptide) for Celiac Disease.

   Lau MS, Mooney PD, White WL, Rees MA, Wong SH, Hadjivassiliou M, Green PHR, Lebwohl B, Sanders DS.

   Am J Gastroenterol. 2018 Aug;113(8):1238-1246

3. Chapter 5: Pre-endoscopy point of care test (Simtomax- IgA/IgG-Deamidated Gliadin Peptide) for coeliac disease in iron deficiency anaemia: diagnostic accuracy and a cost saving economic model.

   Lau MS, Mooney P, White WL, Appleby V, Moreea S, Haythem I, Elias JE, Bundhoo K, Corbett GD, Wong L, Tsai HH, Cross SS, Hebden JM, Hoque S and Sanders DS.

   BMC Gastroenterol. 2016 Sep 15;16:115.

4. Chapter 6: The Role of an IgA/IgG-Deamidated Gliadin Peptide Point-of-Care Test in Predicting Persistent Villous Atrophy in Patients with Celiac Disease on a Gluten-Free Diet.

Lau MS, Mooney PD, White WL, Rees MA, Wong SH, Kurien M, Trott N, Leffler DA, Hadjivassiliou M, Sanders DS.

Am J Gastroenterol. 2017 Dec;112(12):1859-1867.

Oral presentations for published abstracts:

1. BSG Conference 2018: Gluten free diet adherence assessment using CDAT and BIAGI questionnaires in patients with coeliac disease.

   Lau MS, Mooney PD, Rees MA*, et al. Gut* 2018;67: A160-A161.

2. BSG Conference 2017: The Role of an IgA/IgG-Deamidated Gliadin Peptide Point-of-Care Test in Predicting Persistent Villous Atrophy in Patients with Celiac Disease on a Gluten-Free Diet.

   Lau MS, Mooney PD, White WL, et al. *Gut* 2017;66: A9-A10.

3. BSG Conference 2017: A pre-endoscopy point of care test (IgA/IgG-deamidated gliadin peptide) as a case finding tool for coeliac disease in secondary care.

   Lau M, Mooney P, White W*, et al. Gut* 2017;66: A20-A21.

4. BSG Conference 2016: Does the Point of Care Test, Simtomax, Distinguish between Coeliac disease and Non Coeliac Gluten Sensitivity?

   MS Lau, PD Mooney, WL White, et al. Gut 2016;65: Suppl 1 A15.

5. BSG Conference 2016: Does Duodenal Histology Yield any other Diagnoses apart from Coeliac Disease?

   MS Lau, PD Mooney, WL White, et al. Gut 2016;65: Suppl 1 A17-A18.

Poster presentations for published abstracts:

1. BSG Conference 2017: A novel primary care case finding strategy for coeliac disease using a point of care test (IgA/IgG-deamidated gliadin peptide) in community pharmacies.

   Lau M, Sanders D. *Gut* 2017;66: A194-A196.

2. BSG Conference 2016: Does Duodenal Histology Yield any Other Diagnoses for Iron Deficiency Anaemia apart from Coeliac Disease?

   MS Lau, PD Mooney, WL White, et al. Gut 2016;65: Suppl 1 A17-A18.

3. BSG Conference 2016**:** The Role of a Point of Care Test, Simtomax, in Predicting Histological Remission in Coeliac Disease on a Gluten Free Diet.

   MS Lau, PD Mooney, WL White, et al. Gut 2016;65: Suppl 1 A166-A167.

Editorial:

Point of care testing for paediatric coeliac disease in the new ESPGHAN era.

Lau MSY, Sanders DS. Rev Esp Enferm Dig. 2017 Oct 30;109:741-742

Review articles:

1. Optimizing the diagnosis of celiac disease.

   Lau MS, Sanders DS. Curr Opin Gastroenterol. 2017 May;33(3):173-180.

2. Improving the detection of coeliac disease.

   Lau MS, Hopper AD, Sanders DS. Practitioner 2016;260(1795):13-17

Press release:

1. ‘Point-of-care-test predicts persistent villous atrophy in celiac disease.’
    - Medscape Gastroenterology News: https://www.medscape.com/viewarticle/887758
    - American College of Gastroenterology (ACG) Smart brief: http://www2.smartbrief.com/servlet/encodeServlet?issueid=7A57BD7F-BA80-4ED5-A152-1ACB55D08309&sid=ff07bbec-d693-4180-86b9-615604a9ba43
    - Reuters Health (US)

2. ‘Simtomax Point-of-care test effective for differentiating between celiac disease and nonceliac gluten sensitivity.’
    - Haelio Gastroenterology Meeting news coverage: https://www.healio.com/gastroenterology/malabsorption/news/online/%7B983a81cd-6c66-453d-8ef5-37894432a62d%7D/simtomax-point-of-care-test-effective-for-differentiating-between-celiac-disease-nonceliac-gluten-sensitivity