**Name of Journal:** *World Journal of Gastroenterology*

**Manuscript NO:** 52299

# **Manuscript Type:** META-ANALYSIS

# **Diagnostic and clinical significance of antigen-specific pancreatic antibodies in inflammatory bowel diseases: A meta-analysis**

# Gkiouras K *et al*. Anti-GP2 for Crohn’s diagnosis: A meta-analysis

# Konstantinos Gkiouras, Maria G Grammatikopoulou, Xenophon Theodoridis, Eirini Pagkalidou, Evangelia Chatzikyriakou, Anna G Apostolidou, Eirini I Rigopoulou, Lazaros I Sakkas, Dimitrios Petrou Bogdanos

**Konstantinos Gkiouras, Maria G Grammatikopoulou, Xenophon Theodoridis, Lazaros I Sakkas, Dimitrios Petrou Bogdanos,** Department of Rheumatology and Clinical Immunology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Biopolis, Larissa GR41110, Greece

**Konstantinos Gkiouras, Maria G Grammatikopoulou, Xenophon Theodoridis, Evangelia Chatzikyriakou,** Faculty of Medicine, School of Health Sciences, Aristotle University of Thessaloniki, University Campus, Thessaloniki GR54124, Greece

**Eirini Pagkalidou,** Laboratory of Hygiene, Social and Preventive Medicine and Medical Statistics, Faculty of Medicine, School of Health Sciences, Aristotle University of Thessaloniki, University Campus, Thessaloniki GR54124, Greece

**Maria G Grammatikopoulou, Anna G Apostolidou,** Department of Nutritional Sciences and Dietetics, School of Health Sciences, International Hellenic University, Sindos Campus, Thessaloniki GR57400, Greece

**Evangelia Chatzikyriakou,** Laboratory of Clinical Neurophysiology, AHEPA University Hospital, Faculty of Medicine, Aristotle University of Thessaloniki, University Campus, Thessaloniki GR54124, Greece

**Eirini I Rigopoulou,** Department of Medicine and Research Laboratory of Internal Medicine, University Hospital of Larissa, Biopolis, Larissa GR41110, Greece

**Dimitrios Petrou Bogdanos,** Division of Transplantation, Immunology and Mucosal Biology, MRC Centre for Transplantation, King's College London Medical School, London GR41110, United Kingdom

**Author contributions:** Gkiouras K and Bogdanos DP designed research; Gkiouras K contributed to data acquisition, analyzed and interpreted data, drafting the article, final approval; Grammatikopoulou MG contributed to acquisition of data, interpreted data, drafting the manuscript, final approval; Theodoridis X contributed to quality assessment, interpreted data, revising the article, final approval; Pagkalidou E contributed to supervision of the statistical analyses, final approval; Chatzikyriakou E contributed to quality assessment of data, final approval; Apostolidou AG contributed to data acquisition, final approval; Rigopoulou EI and Sakkas LI contributed to data interpretation, drafting manuscript parts, final approval; Bogdanos DP contributed to conception and design of the study, acquisition of data, supervision of all analyses, critical revision, final approval.

**Corresponding author: Dimitrios Petrou Bogdanos, MD, PhD, Assistant Professor,** Department of Rheumatology and Clinical Immunology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Biopolis, PO Box 1425, Larissa GR41110, Greece. bogdanos@med.uth.gr

**Received:** November 1, 2019

**Revised:** December 19, 2019

**Accepted:** January 2, 2020

**Published online:** January 14, 2020

**Abstract**

BACKGROUND

Non-invasive criteria are needed for Crohn’s disease (CD) diagnosis, with several biomarkers being tested. Results of individual diagnostic test accuracy studies assessing the diagnostic value of pancreatic autoantibodies-to-glycoprotein-2 (anti-GP2) tests for the diagnosis of CD appear promising.

AIM

To systematically review and meta-analyze evidence on the diagnostic accuracy of anti-GP2 tests in patients with suspected/confirmed CD.

METHODS

An electronic search was conducted on PubMed, Cochrane-CENTRAL and grey literature (CRD42019125947). The structured research question in PICPTR format was “Population” including patients with symptoms akin to CD, the “Index test” being anti-GP2 testing, the “Comparator” involved standard CD diagnosis, the “Purpose of test” being diagnostic, “Target disorder” was CD, and the “Reference standard” included standard clinical, radiological, endoscopical, and histological CD diagnostic criteria. Quality was assessed with the Quality Assessment of Diagnostic Accuracy Studies-2 tool and hierarchical models were employed to synthesize the data.

RESULTS

Out of 722 studies retrieved, 15 were meta-analyzed. Thirteen studies had industry-related conflicts-of-interest, and most included healthy donors as controls (spectrum bias). For the combination of IgA and/or IgG anti-GP2 test, the summary sensitivity was 20% (95% confidence interval: 10%–29%) at a median specificity of 97%. If the test was applied in 10000 suspected patients, 9669 would be true negatives and in 26, the diagnosis would be missed. In this hypothetical cohort, the anti-GP2 would fail to produce a diagnosis for 81.3% of the positive cases. Low summary points of sensitivity and high specificity were estimated for the IgG or IgA anti-GP2 test. Analogous results were observed when the analyses were restricted using specific cut-offs, or when ulcerative colitis patients were used as comparators.

CONCLUSION

Anti-GP2 tests demonstrate low sensitivity and high specificity. These results indicate that caution is required before relying on its diagnostic value. Additionally, the need for improving the methodology of diagnostic test accuracy studies is evident.

**Key words:** Inflammatory bowel disease; Gastrointestinal disease; Evidence-based diagnosis; Sensitivity; Specificity; Ulcerative colitis; Conflicts of interest; Meta-regression; Industry bias

Gkiouras K, Grammatikopoulou MG, Theodoridis X, Pagkalidou E, Chatzikyriakou E, Apostolidou AG, Rigopoulou EI, Sakkas LI, Bogdanos DP. Diagnostic and clinical significance of antigen-specific pancreatic antibodies in inflammatory bowel diseases: A meta-analysis. *World J Gastroenterol* 2020; 26(2): 246-265

**URL:** https://www.wjgnet.com/1007-9327/full/v26/i2/246.htm

# **DOI:** https://dx.doi.org/10.3748/wjg.v26.i2.246

**Core tip:** The majority of individual studies assessing the diagnostic accuracy of autoantibodies for anti-glycoprotein 2 (anti-GP2) for Crohn’s disease (CD) diagnosis either include asymptomatic participants, or patients with symptoms not akin to CD. Most studies carry industry-related conflicts-of-interest, employing non-blinded evaluation of their assays and CD diagnosis preceding anti-GP2 testing. The pooled analyses performed herein using only symptomatic patients as controls, revealed high heterogeneity and low diagnostic accuracy of the anti-GP2, demonstrating low sensitivity and high specificity. Based on the pooled sensitivity and specificity of the anti-GP2 for CD diagnosis, they do not appear to attain the characteristics to be used *per se* as a proper non-invasive diagnostic tool.

**INTRODUCTION**

Pancreatic secretory granule membrane glycoprotein 2 (GP2) consists of a 78  kDa glycoprotein[1]. GP2 is synthesized by the acinus cells[1] in the pancreas, and is considered today as the main target of pancreatic autoantibody[2,3]. Recent data indicate that GP2 is a specific receptor on microfold (M) cells of intestinal Peyer's patches[4–6], which consist of the original inflammation site in Crohn’s Disease (CD)[2]. With autoreactive responses being important effectors of immune-mediated inflammation, triggering overt inflammatory bowel diseases (IBD)[7], autoantibodies-to-glycoprotein-2 (anti-GP2) have recently been suggested as possible diagnostic markers of CD.

Today, CD differential diagnosis is based on standard clinical, radiological, endoscopical and histological criteria[8,9], and a need for less invasive diagnostic tools has been highlighted, especially given the great number of patients with clinical features mimicking CD[10]. This is why recently, many diagnostic test accuracy (DTA) studies have been conducted, assessing the specificity and sensitivity of various biomarkers against standard CD diagnostic procedures[11], including the anti-GP2.

Despite the fact that a plethora of DTA studies has recently been conducted assessing the sensitivity and specificity of the GP2 autoantibodies for CD’s differential diagnosis, synthesis of these studies in the form of a systematic review and meta-analysis would undoubtedly produce more valid results, as compared to individual studies, aiding evidence-based diagnosis[12]. Meta-analyses of DTA studies are important to obtain more valid, summary estimates of the diagnostic accuracy of an index test[13]. One such meta-analysis investigating the diagnostic accuracy of anti-GP2 for CD was published during the year 2017[14], missing however, many of the DTA studies published since then. Additionally, this specific meta-analysis[14] also exhibited few methodological shortcomings, like the improper inclusion of healthy controls in the samples analyzed, although for DTA studies, only patients with symptoms akin to the disease investigated are to be used[15–17].

Given the need for less invasive diagnostic tests (preferably serological) to be used in individuals with clinical suspicion of CD, while identifying the literature gap as per relevant state-of-the-art systematic reviews, the aim of the present systematic review and meta-analysis was to synthetize evidence examining the diagnostic accuracy of anti-GP2 tests in patients with suspected or confirmed CD. The PPPICPTR[18] an adapted PICO for systematic reviews of DTA was applied. In further detail, the PICPTR of the study was Population including patients with gastrointestinal symptoms akin to CD, with the Index test being positive anti-GP2 testing, the Comparator being standard CD diagnosis, the Purpose of test was diagnostic, with the Target disorder being CD, and the Reference standard included the standard clinical, radiological, endoscopical and histological criteria for CD diagnosis[18].

**MATERIALS AND METHODS**

***Literature search***

Reporting standards are based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses of Diagnostic Test Accuracy[19,20]. The protocol of the present systematic review was registered at PROSPERO (CRD42019125947).

A systematic search was conducted using the PubMed and Cochrane CENTRAL databases, until February, 28 2019. The grey literature and websites of companies manufacturing anti-GP2 kits were also explored for possible references using the specific tests. The keywords used in the searches included (anti-glycoprotein 2 antibody), (autoantibodies to glycoprotein 2), (anti-gp2), (autoantibodies), (Crohn’s disease), with a combination of MeSH terms wherever possible. In particular, Table 1 details the search strategy used for PubMed and Cochrane-CENTRAL. The keyword ‘anti-glycoprotein’ was used for searches within grey literature sources (Open Grey and National Technical Information Service) and on websites of anti-GP2 manufacturers (Euroimmun, GA Generic Assays, Thermo Fisher, and AMS Biotechnology). Studies were assessed for eligibility independently and in duplicate, by three researchers (Gkiouras K, Grammatikopoulou MG and Bogdanos DP), and any disagreements were resolved by consensus.

***Inclusion and exclusion criteria***

We imposed no restrictions on the age of the study population, language, or the quality of retrieved DTA studies. Studies assessing anti-GP2 levels to diagnose CD in patients with relevant clinical features were selected. Additionally, studies assessing anti-GP2 levels among IBD patients were also considered eligible. The reference standard to verify CD diagnosis was the standard clinical, radiological, endoscopical and histological criteria for CD diagnosis[8,9,21–25].

However, studies were excluded when (1) based on animals or non-human samples; (2) not providing sufficient data to construct a 2 × 2 table; (3) presenting duplicate data already reported in other manuscripts; and (4) not reporting the reference CD diagnostic criteria. When two publications had been identified as using overlapping populations, they were counted as a single study[26].

***Data extraction***

The main outcomes of interest involved sensitivity, specificity and the diagnostic odds ratio[27,28].

Data were extracted by Gkiouras K and Grammatikopoulou MG on prespecified data extraction sheets for DTA studies, as suggested by the Joanna Briggs Institute[29], which were then checked by Bogdanos DP. For studies reporting sensitivity, specificity, positive and negative predictive values and a total number of included patients, 2 × 2 tables of true positives (TP), false positives, false negatives, and true negatives (TN) were calculated, following the instructions of the Oxford Centre for Evidence-Based Medicine[30].

***Quality assessment***

Quality of included studies was assessed independently and in duplicate by two reviewers (Theodoridis X and Chatzikyriakou E), using the criteria of the Quality Assessment of Diagnostic Accuracy Studies-2 tool[31].

***Meta-analysis***

Given the great variability regarding the cut-offs used to define disease status in the primary studies[32,33], the hierarchical summary receiver operating characteristic (HSROC) model[34,35] was employed to synthesize data. SROC curves were constructed, but considering that a summary point of sensitivity or specificity among studies using mixed thresholds would be clinically uninterpretable, we chose to estimate summary sensitivity at its median specificity, based on the SROC curves[15,33]. When more than three primary studies reported similar cut-offs, the analysis was repeated with the hierarchical Bivariate model in order to obtain summary points of sensitivity and specificity[35].

Furthermore, heterogeneity was assessed statistically by including covariates in the HSROC model (meta-regression). Heterogeneity is summarized with the relative diagnostic odds ratios (RDOR) along with their 95% confidence intervals (95%CI). The included covariates involved: Source of funding [state *vs* other (including private or not stated)], diagnostic kit industry conflicts-of-interest (COI) [industry (studies either reporting funding from diagnostic kit manufacturers, or having authors employed in the industry) *vs* other (lack of apparent industry-related COI)], the assay used for detecting autoantibodies (enzyme-linked immunosorbent assay *vs* indirect immunofluorescence), the manufacturers of the anti-GP2 kits (Generic assays *vs* other), blinding of the assay (lacking or not stated *vs* yes), recruitment of consecutive patients (no/not stated *vs* yes), and the percentage of female participants categorized as ≥ 50% *vs* < 50%.

When the complete HSROC models failed to converge and/or returned unstable parameters they were simplified with the symmetric HSROC model or the HSROC model with fixed accuracy, as previously described[36]. Similarly, when the Bivariate model returned unstable parameters, the analysis was repeated with univariate random effects models (UREM), as previously proposed[36]. The fit of the models was assessed with the -2 Loglikelihood test[35]. All analyses were repeated twice, once including DTA studies reporting results from CD cases against all patients with relevant symptoms, and the second time including studies reporting CD cases against ulcerative colitis (UC) cases only. In the analyses combining the result of IgA and/or IgG positive antibodies, studies were included only when reporting relevant results in detail.

Estimates of sensitivities and specificities derived for specific cut-off values were expressed as natural frequencies and summarized in a table[37]. Since the majority of included studies were based in Europe, Germany in particular, and had a case-control design, the estimation of CD prevalence would not have been precise. Subsequently, the prevalence rates used herein were extracted from a recent systematic review[38]. The prevalence rate used was 322 per 100000[38] in a hypothetical cohort of 10000 suspected patients. This figure was selected based on its efficiency to produce logical natural frequencies.

Statistical analyses were carried with the SAS PROC NLMIXED procedure and/or the MetaDAS macro[39] on SAS software (SAS Institute Inc., Cary, NC, United States) and the plots were developed with RevMan[40]. The statistical methods used in this study were reviewed by Anna-Bettina Haidich, Associate Professor of Medical Statistics and Epidemiology in Aristotle University of Thessaloniki.

**RESULTS**

Out of 722 DTA studies retrieved in total, 18[41-58] fulfilled the systematic review’s protocol criteria. Figure 1 details the selection process of the primary DTA studies. As three studies[44,45,53] did not assess total anti-GP2 but different anti-GP2 isoforms, these were excluded from the meta-analyses, leaving a total of 15 studies[41-43,54–58,46–52].

***Study characteristics and quality assessment of studies***

Table 2 details the characteristics of the 18 primary DTA studies included in the systematic review. All retrieved studies involved full-text articles, except from the one by Op De Beéck *et al*[48], which was in Letter format. None of the studies reported information on the ethnicity of the samples. The Bonaci-Nicolic *et al*[42] study was the only one lacking ethical permission disclosure, whereas the DTA by Op De Beéck *et al*[48] had reported related ethics in a previous study using part of the same sample[59]. Cummings and associates[44] were the only ones recruiting unrelated participants, whereas seven DTA studies in total included children in their samples. Only five studies assured blinding the assays[43–45,49,51]. Cut-offs used to define positivity in IgA or IgG varied greatly, ranging from 3.7 U/dL to 71.75 U/dL for specific GP2 isoforms.

The quality assessment summary using the QUADAS–2 tool[31] is presented in Figure 2. Risk of bias for the index test was generally unclear since, in most studies, it was unclear if the thresholds used had been prespecified by the kit’s manufacturer, or were study-derived[26]. Additionally, many primary DTA studies failed to report whether the anti-GP2 assay was performed with the results of the CD diagnosis being blind[26].

***Meta-analysis of the diagnostic accuracy of anti-GP2 (IgG) for CD***

A total of 15 studies were included in the pooled analyses for evaluation of the diagnostic accuracy of the anti-GP2 IgG (Figure 3), including a pooled sample of 4365 patients, with 665 of them being CD cases and 3700 forming the controls group. The diagnostic sensitivity of the anti-GP2 (IgG) for CD ranged between 10% to 43% (Figure 3A), and the specificity ranged from 80 to 100% (Figure 3B). The summary SROC curve is presented in Figure 3C, indicating that on the median specificity of 93%, summary sensitivity reached 27% (95%CI: 20%–34%). With the UREM models (seven DTA studies), it was estimated that at the cut-off level of 20 U/mL, summary sensitivity reached 22% (95%CI: 15%–30%) and specificity was calculated at 93% (95%CI: 91%–95%). At the cut-off of 15 U/mL (three studies, Bivariate model), summary sensitivity was 28% (95%CI: 16%–43%) and specificity reached 92% (95%CI: 84%–96%).

Forest plots of sensitivity and specificity and the summary SROC curve for the diagnostic accuracy of anti-GP2 in patients with CD against those with UC (14 studies, total patients: 3947; CD cases: 640; UC cases: 3307) are presented in Figures 3D-F. A potential outlier study, the one conducted by Bonaci-Nicolic and associates[42], was identified from the forest plot and the space of the SROC curve, indicating the need for refitting the HSROC model accordingly, after excluding this study. Based on the -2 Loglikelihood test (*P* < 0.001), the remaining analyses were carried out without this DTA study[42]. Based on the HSROC model, on the median specificity of 93% summary sensitivity was 30% (95%CI: 24%–36%). With the UREM models, using the cut-off of 20 U/mL (six studies), summary sensitivity was calculated at 24% (95%CI: 17%–33%) and the specificity at 93% (95%CI: 90%–96%). At the cut-off limit of 15 U/mL, summary sensitivity reached 28% (95%CI: 16%–43%) and specificity was estimated at 90% (95%CI: 84%–94%).

***Meta-analysis of the diagnostic accuracy of anti-GP2 (IgA) for CD***

A total of 14 studies were included in the pooled analysis for the diagnostic accuracy of anti-GP2 IgA, involving 3914 patients in total (CD cases: 380; Control cases: 3534). The reported diagnostic sensitivity ranged from 3% to 37% (Figure 4A) and specificity between 75% to 100% (Figure 4B). Using the HSROC model, the estimated sensitivity on the SROC curve was estimated at 15% (95%CI: 12%–18%) and median specificity reached 97% (Figure 4C). Using the cut-off value of 20 U/mL (seven studies, bivariate model), summary sensitivity was 16% (95%CI: 9%–26%) and specificity was calculated at 96% (95%CI: 86%-99%).

When UC cases were used as the only comparators (Figures 4D and E**;** Total patients: 3497; CD cases: 324; UC cases: 3173) the estimated sensitivity on the SROC curve was 11% (95%CI: 3%–20%) at the median specificity of 98% (Figure 4F). However, when the analysis was restricted to studies reporting results at the cut-off of 20 U/mL (eight studies, UREM model), pooled summary sensitivity was calculated at 15% (95%CI: 10%–22%) and specificity reached 98% (95%CI: 96%–99%).

***Meta-analysis of the diagnostic accuracy of anti-GP2 (IgA and/or IgG) for CD***

A total of five studies were meta-analyzed during the assessment of the diagnostic accuracy of anti-GP2 IgA and/or IgG antibodies, involving a total of 1693 patients (CD cases: 243; Control cases: 1450). The reported diagnostic sensitivity of the anti-GP2 antibody (IgA and/or IgG) for CD ranged between 10% and 34% (Figure 5A), and the reported specificity ranged from 81% to 98% (Figure 5B). The estimated sensitivity was calculated at 20% (95%CI: 10%–29%) at a median specificity of 97% (Figure 5C). At the cut-off value of 20 U/mL (three studies, UREM model), pooled summary sensitivity reached 22% (95%CI: 12%–39%) and pooled summary specificity was computed at 93% (95%CI: 80%–98%).

When UC cases were used as comparators against patients with CD (Figures 5D and E; Total patients: 1541; CD cases: 203; UC cases: 1338), the estimated sensitivity was 20% (95%CI: 4%–35%) at the median specificity of 97% (Figure 5F).

***Investigation of heterogeneity***

Meta-regression analyses were conducted to explore possible sources of heterogeneity. The results (Table 3) revealed that the assay used to detect the anti-GP2 antibodies was linked with the accuracy of anti-GP2 IgG, both in the pooled patient analysis (RDOR = 4.25, 95%CI: 1.26–14.37), as well as in the analysis using UC cases as comparators (RDOR = 3.28, 95%CI: 1.33–8.09). However, these results should be interpreted with caution due to the small number of studies included. The rest of the variables failed to demonstrate significant associations. In the analyses for the anti-GP2 IgA antibodies with the CD *vs* the UC cases, the models failed to converge, or returned unstable parameters even with the use of alternative models (see aforementioned Meta-analysis paragraph).

***Real-life scenario modeling the present findings***

To “measure” the exact effects of using the anti-GP2 assays for the diagnosis of CD according to the results herein and in a hypothetical pragmatic scenario[60], we used the recent data on IBD prevalence[38] (Table 4). When using the combination of IgG and/or IgA anti-GP2 for the diagnosis of CD in a hypothetical cohort of 10000 suspected patients, 9669 will be the TN cases and for 26 CD diagnosis will missed (false negatives) although suffering from CD. In contrary, in the same cohort, 32 patients will suffer from CD and the test will correctly identify only 6 CD cases (TP). Analogous results will be observed if this test is implemented with a cut-off value of 20 U/dL, or in patients suspected only for CD *vs*. UC. Likewise, in the analyses of CD *vs* either all symptomatic patients or UC cases only, using the IgG or IgA anti-GP2 tests would result in similarly increased TN and decreased TP cases.

**DISCUSSION**

The present meta-analysis of DTA studies revealed that the anti-GP2 have a low diagnostic accuracy (low sensitivity and high specificity) in detecting CD true positive cases. In contrast to the published primary DTA research, when all relevant DTA studies were pooled together, the autoantibodies did not appear sensitive enough to detect true positive CD cases.

According to Lalkhen *et al*[61] the ideal diagnostic test is never positive in a disease-free patient, and never negative in a patient with the disease. With the low sensitivity and high specificity demonstrated herein, it appears that the anti-GP2 fall short in the diagnostic accuracy of CD. According to the rule of thumb suggested by Power[62], in a useful test, the sum of sensitivity + specificity must exceed 1.5, ideally reaching 2.0. In none of the analyses performed herein did the sum of sensitivity + specificity exceed 1.5. When high specificity is detected, the problem of overdiagnosis[62] becomes pivotal. However, low sensitivity and high specificity are ideal characteristics of a screening tool, rather than a diagnostic one[63].

Although different isoforms of the anti-GP2 have been identified since the beginning of the century[64], it is only until very recently that the diagnostic potential of all four isoforms was investigated and compared[53]. According to some researchers, anti-GP2 isoforms 1 and 4 are considered as the best serological markers for CD diagnosis, superior even to the anti-saccharomyces cerevisiae antibodies (ASCA), which are routinely used, despite their poor specificity and insufficient sensitivity[44]. Among the included DTA studies, Papp *et al*[49] reported the use of two different enzyme-linked immunosorbent assay methods employing recombinant human GP2 identified as isoform 4. Degehardt and associates[45] made the distinction between two different isoforms of GP2 synthesized in the pancreas, the larger isoform alpha (analogous to isoforms 1 and 3), and the shorter beta form (analogous to isoforms 1 and 3). However, it is not within the scope of the present paper to further discuss the implications related to antibody reactivities against distinct GP2 isoforms. It should be noted however, that due to the different reported isoforms in the included DTA studies and the small number of studies reporting reactivity against different GP2 isoforms (three)[44,45,53], no analyses could be performed to compare the diagnostic accuracy of different anti-GP2 subtypes. However, when more DTA studies of good methodological quality are published using GP2 isoforms, the diagnostic accuracy of the anti-GP2 might be improved in the respective pooled analyses compared to the total anti-GP2 which was evaluated herein.

One important methodological issue identified in the study involves the inclusion of already diagnosed patients, without securing blinding of the index text. The majority of DTA studies on anti-GP2 were performed on already diagnosed CD patients and only five[43–45,49,51] reported blinding of the assays. This issue results in two forms of bias, being: (1) partial verification bias[13], as only patients with a positive result on the index test (anti-GP2 assay) have actually undergone the reference standard test for CD diagnosis (although in reverse order); and (2) test review bias[13], as the results of the reference standard are known to reviewers who interpret the index test. Another important limitation of most primary DTA studies involves the inclusion of healthy controls in their samples, either in the form of healthy donors, or as outpatients. This error was even detected in a recently published meta-analysis of anti-GP2 DTA studies[14]. The inclusion of healthy controls, or of patients with a disease having symptoms not akin to CD[15–17] appears to form a systematic error, exhibited by most primary DTA research and has been reported to result in spectrum bias and overestimation of the diagnostic accuracy[15,65]. This was corrected in the present analyses, where CD cases were only compared either against symptomatic patients, or against patients with UC.

When compared to the recently published meta-analysis[14] of anti-GP2 diagnostic accuracy for CD, the sensitivity and specificity previously reported is similar to the one calculated herein, despite pooling healthy controls together in the analyses. Still, authors of that meta-analysis[14] acknowledge several of the limitations of applying the anti-GP2 assay as a diagnostic tool and suggest its use for the differentiation of CD patients from controls, although their definition of controls for DTA studies appears to be arbitrary. According to Al Fattani *et al*[66], evidence from well-designed thorough systematic reviews indicate the importance of attaining a correct methodological design in DTA studies.

Another issue of concern and possible source of bias that may partly explain the systematic error of using healthy controls, or patients with irrelevant symptoms in the control groups, involves the industry-related COI demonstrated among most primary DTA studies. With 13[41,43-45,48,49,51-56,58] out of 18 primary studies included in the systematic review either reporting direct funding by diagnostic kit manufacturers, receiving the kits for gratis, or including authors with kit-industry affiliations, this may partially explain the methodological mistakes detected in most DTA studies, either in the form of guidance throughout the study’s implementation, or in the form of statistical or interpretation advice provided by the kit industry. Although comparison between DTA studies with industry-related COI *vs* those lacking any apparent industry-related COI did not differ in terms of anti-GP2 accuracy, further investigation is needed to examine industry-related COI in DTA studies. It is well known that financial competing interests in industry-sponsored research often introduce bias into study design, analyses and interpretation of findings[67], as observed herein. Fairly recently, it was suggested that in several cases, the industry might be involved in overdiagnosis due to underlying financial profits[68,69]. Dakubo *et al*[70] was the first to identify industry financial interests as a major cause of overdiagnosis, however, to our knowledge, COI in DTA research have never been evaluated, nor has the overall influence of the industry. As many of the primary studies included herein failed to report any funding source[42,44,53], it is highly likely that some might have received the diagnostic kits for gratis by the industry without reporting it, or without disclosing relevant academia-industry funding.

The variety of thresholds used in most studies to identify TP CD cases is yet another issue of concern. Given the high number of industry-related COI demonstrated in the primary DTA studies, one might argue that cut-offs are defined arbitrary, based on the expected TP prevalence in order to fulfill the minimum of positive and negative cases set by the College of American Pathologists[71,72]. Thus, it might be wise to report the diagnostic test thresholds in advance, possibly in the form of a published protocol, before initiating a DTA study.

In the present study we explored many potential sources of heterogeneity extensively and only the assay used to detect the anti-GP2 antibodies was associated with heterogeneity affecting the diagnostic accuracy of anti-GP2 IgG. The high degree of clinical heterogeneity exhibited in the primary DTA studies limits the possibility of making strong conclusions regarding the diagnostic performance of anti-GP2 antibodies. Another issue which needs to be taken into account is the performance of this test in combination with other non-invasive tests, such as fecal calprotectin and the ASCA, which are routinely used for the investigation of cases with a clinical suspicion of CD[73–75].

Undoubtedly, diagnostic tests can aid practitioners in the diagnostic process[76]. It appears that identifying a sensitive test without misclassification of many false positives remains a challenge[77], as most tests are imperfect and can only adjust disease probability[12]. Tests with low sensitivity and high specificity, like the anti-GP2, are better for population screening rather than for diagnosing patients[62,63]. Additionally, it appears that given the methodological pitfalls demonstrated in most anti-GP2 DTA research, high quality DTA cohort studies are required, enrolling consecutive patients, presenting clinical and laboratory features akin to CD, where both the assay and the reference diagnosis will be performed in a double-blind manner, preferably without the industry being involved at any step of the process, other than providing the relevant kits. As per CD diagnosis, we would have to agree with the European Crohn and Colitis Organization[78] that based on the currently available data, serological tests should be used as diagnostic adjuvants in parallel to colonoscopy.

In recap, CD differential diagnosis is important[79,80]. Despite the high accuracy reported in individual primary DTA studies, and the gaining residence of the anti-GP2 use in CD diagnosis, the present systematic review and meta-analysis revealed that when the anti-GP2 are used as a proxy for the diagnosis of CD the results should be interpreted with caution, due to its relatively low sensitivity and high specificity.

**Article Highlights**

***Research background***

Non-invasive criteria are needed for Crohn’s disease (CD) diagnosis, with several biomarkers being tested, including the pancreatic autoantibodies-to-glycoprotein-2 (anti-GP2).

***Research motivation***

Results of individual diagnostic test accuracy (DTA) studies assessing the diagnostic value of the anti-GP2 for the diagnosis of CD appear promising, however, a systematic review and meta-analysis of the studies is still lacking.

***Research objectives***

The aim of the present systematic review and meta-analysis was synthesize all evidence on the diagnostic accuracy of anti-GP2 tests in patients with suspected/confirmed CD.

***Research methods***

An electronic search was conducted on Medline, Cochrane-CENTRAL and grey literature. Quality was assessed with the Quality Assessment of Diagnostic Accuracy Studies-2 tool and hierarchical models were employed to synthesize the data. The hierarchical summary receiver operating characteristic (HSROC) model was employed to synthesize data. SROC curves were constructed and since a summary point of sensitivity or specificity with studies using mixed thresholds would be clinically uninterpretable, the summary sensitivity was estimated at its median specificity, based on the SROC curves. Heterogeneity was assessed statistically by including covariates in the HSROC model (meta-regression) and was summarized with the Relative Diagnostic Odds Ratios.

***Research results***

Out of 722 studies retrieved, 15 were meta-analyzed. Thirteen studies had industry-related conflicts-of-interest, and most included healthy donors as controls. For the combination of IgA and/or IgG anti-GP2 test, the summary sensitivity was 20% at a median specificity of 97%.

***Research conclusions***

The anti-GP2 demonstrated low sensitivity and high specificity. These results indicate caution before relying on its diagnostic value. However, the anti-GP2 appear to attain all characteristics of a screening tool rather than a diagnostic one. Therefore, based on the available evidence, the use of the anti-GP2 for CD diagnosis is not warranted. Furthermore, overall quality of DTA studies appears low, with many carrying industry-related, spectrum, test-review and partial verification bias. Thus, the need for improving the methodology of DTA studies is evident.

***Research perspectives***

The majority of DTA studies are lacking a quality design and should be synthetized with caution. Future research should assess differences between industry-funded and non-industry funded DTA studies.

**ACKNOWLEDGEMENTS**

All authors appreciate the critical review provided by Assistant Professors Dr. Anna-Bettina Haidich and Dr. Stavros Kalogiannis, as well as the support offered by our friend and colleague Dr. Meletios P Nigdelis, in lending us his voice for the audio core tip! The present study was presented at the 12th International Congress on Autoimmunity (2020) and the 11th Greek Immunology Congress (2019), both held in Athens, Greece.

**REFERENCES**

1 **Hoops TC**, Ivanov I, Cui Z, Colomer-Gould V, Rindler MJ. Incorporation of the pancreatic membrane protein GP-2 into secretory granules in exocrine but not endocrine cells. *J Biol Chem* 1993; **268**: 25694-25705 [PMID: 7503984]

2 **Roggenbuck D**, Reinhold D, Werner L, Schierack P, Bogdanos DP, Conrad K. Glycoprotein 2 antibodies in Crohn's disease. *Adv Clin Chem* 2013; **60**: 187-208 [PMID: 23724745 DOI: 10.1016/B978-0-12-407681-5.00006-4]

3 **Conrad K**, Hausdorf G, Feist E, Reinhold D, Jungblut P, Porstmann T, Laass M, Henker J, Roggenbuck D. Identification of GP2 as the major autoantigen of pancreatic autoantibodies. Proceedings of the 6th Congress on Autoimmunity; 2008 Sep 9-13; Porto, Portugal. Immunotherapy, 2008.

4 **Hase K**, Kawano K, Nochi T, Pontes GS, Fukuda S, Ebisawa M, Kadokura K, Tobe T, Fujimura Y, Kawano S, Yabashi A, Waguri S, Nakato G, Kimura S, Murakami T, Iimura M, Hamura K, Fukuoka S, Lowe AW, Itoh K, Kiyono H, Ohno H. Uptake through glycoprotein 2 of FimH(+) bacteria by M cells initiates mucosal immune response. *Nature* 2009; **462**: 226-230 [PMID: 19907495 DOI: 10.1038/nature08529]

5 **Terahara K**, Yoshida M, Igarashi O, Nochi T, Pontes GS, Hase K, Ohno H, Kurokawa S, Mejima M, Takayama N, Yuki Y, Lowe AW, Kiyono H. Comprehensive gene expression profiling of Peyer's patch M cells, villous M-like cells, and intestinal epithelial cells. *J Immunol* 2008; **180**: 7840-7846 [PMID: 18523247 DOI: 10.4049/jimmunol.180.12.7840]

6 **Ohno H**, Hase K. Glycoprotein 2 (GP2): grabbing the FimH bacteria into M cells for mucosal immunity. *Gut Microbes* 2010; **1**: 407-410 [PMID: 21468225 DOI: 10.4161/gmic.1.6.14078]

7 **Roggenbuck D**, Reinhold D, Schierack P, Bogdanos DP, Conrad K, Laass MW. Crohn's disease specific pancreatic antibodies: clinical and pathophysiological challenges. *Clin Chem Lab Med* 2014; **52**: 483-494 [PMID: 24231127 DOI: 10.1515/cclm-2013-0801]

8 **Stange EF**, Travis SP, Vermeire S, Beglinger C, Kupcinkas L, Geboes K, Barakauskiene A, Villanacci V, Von Herbay A, Warren BF, Gasche C, Tilg H, Schreiber SW, Schölmerich J, Reinisch W; European Crohn's and Colitis Organisation. European evidence based consensus on the diagnosis and management of Crohn's disease: definitions and diagnosis. *Gut* 2006; **55** Suppl 1: i1-15 [PMID: 16481628 DOI: 10.1136/gut.2005.081950a]

9 **IBD Working Group of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition**. Inflammatory bowel disease in children and adolescents: recommendations for diagnosis--the Porto criteria. *J Pediatr Gastroenterol Nutr* 2005; **41**: 1-7 [PMID: 15990620 DOI: 10.1097/01.MPG.0000163736.30261.82]

10 **Marlicz W**, Skonieczna-Żydecka K, Dabos KJ, Łoniewski I, Koulaouzidis A. Emerging concepts in non-invasive monitoring of Crohn's disease. *Therap Adv Gastroenterol* 2018; **11**: 1756284818769076 [PMID: 29707039 DOI: 10.1177/1756284818769076]

11 **Castiglione F**, Mainenti PP, De Palma GD, Testa A, Bucci L, Pesce G, Camera L, Diaferia M, Rea M, Caporaso N, Salvatore M, Rispo A. Noninvasive diagnosis of small bowel Crohn's disease: direct comparison of bowel sonography and magnetic resonance enterography. *Inflamm Bowel Dis* 2013; **19**: 991-998 [PMID: 23429465 DOI: 10.1097/MIB.0b013e3182802b87]

12 **Bianchi MT**, Alexander BM. Evidence based diagnosis: does the language reflect the theory? *BMJ* 2006; **333**: 442-445 [PMID: 16931846 DOI: 10.1136/bmj.38915.558738.55]

13 **Kim KW**, Lee J, Choi SH, Huh J, Park SH. Systematic Review and Meta-Analysis of Studies Evaluating Diagnostic Test Accuracy: A Practical Review for Clinical Researchers-Part I. General Guidance and Tips. *Korean J Radiol* 2015; **16**: 1175-1187 [PMID: 26576106 DOI: 10.3348/kjr.2015.16.6.1175]

14 **Deng C**, Li W, Li J, Zhang S, Li Y. Diagnostic value of the antiglycoprotein-2 antibody for Crohn's disease: a PRISMA-compliant systematic review and meta-analysis. *BMJ Open* 2017; **7**: e014843 [PMID: 28601823 DOI: 10.1136/bmjopen-2016-014843]

15 **Leeflang MM**, Deeks JJ, Gatsonis C, Bossuyt PM; Cochrane Diagnostic Test Accuracy Working Group. Systematic reviews of diagnostic test accuracy. *Ann Intern Med* 2008; **149**: 889-897 [PMID: 19075208 DOI: 10.7326/0003-4819-149-12-200812160-00008]

16 **Rutjes AW**, Reitsma JB, Di Nisio M, Smidt N, van Rijn JC, Bossuyt PM. Evidence of bias and variation in diagnostic accuracy studies. *CMAJ* 2006; **174**: 469-476 [PMID: 16477057 DOI: 10.1503/cmaj.050090]

17 **Lijmer JG**, Mol BW, Heisterkamp S, Bonsel GJ, Prins MH, van der Meulen JH, Bossuyt PM. Empirical evidence of design-related bias in studies of diagnostic tests. *JAMA* 1999; **282**: 1061-1066 [PMID: 10493205 DOI: 10.1001/jama.282.11.1061]

18 **Bae JM**. An overview of systematic reviews of diagnostic tests accuracy. *Epidemiol Health* 2014; **36**: e2014016 [PMID: 25209601 DOI: 10.4178/epih/e2014016]

19 **McGrath TA**, Alabousi M, Skidmore B, Korevaar DA, Bossuyt PMM, Moher D, Thombs B, McInnes MDF. Recommendations for reporting of systematic reviews and meta-analyses of diagnostic test accuracy: a systematic review. *Syst Rev* 2017; **6**: 194 [PMID: 29017574 DOI: 10.1186/s13643-017-0590-8]

20 **McInnes MDF**, Moher D, Thombs BD, McGrath TA, Bossuyt PM; and the PRISMA-DTA Group, Clifford T, Cohen JF, Deeks JJ, Gatsonis C, Hooft L, Hunt HA, Hyde CJ, Korevaar DA, Leeflang MMG, Macaskill P, Reitsma JB, Rodin R, Rutjes AWS, Salameh JP, Stevens A, Takwoingi Y, Tonelli M, Weeks L, Whiting P, Willis BH. Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies: The PRISMA-DTA Statement. *JAMA* 2018; **319**: 388-396 [PMID: 29362800 DOI: 10.1001/jama.2017.19163]

21 **Hanauer SB**. Inflammatory bowel disease. *N Engl J Med* 1996; **334**: 841-848 [PMID: 8596552 DOI: 10.1056/NEJM199603283341307]

22 **Harbord M**, Annese V, Vavricka SR, Allez M, Barreiro-de Acosta M, Boberg KM, Burisch J, De Vos M, De Vries AM, Dick AD, Juillerat P, Karlsen TH, Koutroubakis I, Lakatos PL, Orchard T, Papay P, Raine T, Reinshagen M, Thaci D, Tilg H, Carbonnel F; European Crohn’s and Colitis Organisation. The First European Evidence-based Consensus on Extra-intestinal Manifestations in Inflammatory Bowel Disease. *J Crohns Colitis* 2016; **10**: 239-254 [PMID: 26614685 DOI: 10.1093/ecco-jcc/jjv213]

23 **Dignass A**, Van Assche G, Lindsay JO, Lémann M, Söderholm J, Colombel JF, Danese S, D'Hoore A, Gassull M, Gomollón F, Hommes DW, Michetti P, O'Morain C, Oresland T, Windsor A, Stange EF, Travis SP; European Crohn's and Colitis Organisation (ECCO). The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Current management. *J Crohns Colitis* 2010; **4**: 28-62 [PMID: 21122489 DOI: 10.1016/j.crohns.2009.12.002]

24 **Lennard-Jones JE**. Classification of inflammatory bowel disease. *Scand J Gastroenterol Suppl* 1989; **170**: 2-6; discussion 16-9 [PMID: 2617184 DOI: 10.3109/00365528909091339]

25 **Levine A**, Koletzko S, Turner D, Escher JC, Cucchiara S, de Ridder L, Kolho KL, Veres G, Russell RK, Paerregaard A, Buderus S, Greer ML, Dias JA, Veereman-Wauters G, Lionetti P, Sladek M, Martin de Carpi J, Staiano A, Ruemmele FM, Wilson DC; European Society of Pediatric Gastroenterology, Hepatology, and Nutrition. ESPGHAN revised porto criteria for the diagnosis of inflammatory bowel disease in children and adolescents. *J Pediatr Gastroenterol Nutr* 2014; **58**: 795-806 [PMID: 24231644 DOI: 10.1097/MPG.0000000000000239]

26 **Taylor KS**, Verbakel JY, Feakins BG, Price CP, Perera R, Bankhead C, Plüddemann A. Diagnostic accuracy of point-of-care natriuretic peptide testing for chronic heart failure in ambulatory care: systematic review and meta-analysis. *BMJ* 2018; **361**: k1450 [PMID: 29785952 DOI: 10.1136/bmj.k1450]

27 **Glas AS**, Lijmer JG, Prins MH, Bonsel GJ, Bossuyt PM. The diagnostic odds ratio: a single indicator of test performance. *J Clin Epidemiol* 2003; **56**: 1129-1135 [PMID: 14615004 DOI: 10.1016/s0895-4356(03)00177-x]

28 **Devillé WL**, Buntinx F, Bouter LM, Montori VM, de Vet HC, van der Windt DA, Bezemer PD. Conducting systematic reviews of diagnostic studies: didactic guidelines. *BMC Med Res Methodol* 2002; **2**: 9 [PMID: 12097142 DOI: 10.1186/1471-2288-2-9]

29 **The Joanna Briggs Institute**. The Joanna Briggs Institute Reviewers’ Manual 2015: The systematic review of studies of diagnostic test accuracy. The Joanna Briggs Institute, 2015: 1-46

30 **Taylor KS**.Tip for data extraction in meta-analysis - 1 - Center for Evidence Based Medicine. 2019 Feb 11 [cited 17 March 2019]. Oxford University [Internet]. Available from: https://www.cebm.net/2019/02/tip-for-data-extraction-in-meta-analysis-1/

31 **Whiting PF**, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JA, Bossuyt PM; QUADAS-2 Group. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011; **155**: 529-536 [PMID: 22007046 DOI: 10.7326/0003-4819-155-8-201110180-00009]

32 **Dukic V**, Gatsonis C. Meta-analysis of diagnostic test accuracy assessment studies with varying number of thresholds. *Biometrics* 2003; **59**: 936-946 [PMID: 14969472 DOI: 10.1111/j.0006-341X.2003.00108.x]

33 **Takwoingi Y**, Riley RD, Deeks JJ. Meta-analysis of diagnostic accuracy studies in mental health. *Evid Based Ment Health* 2015; **18**: 103-109 [PMID: 26446042 DOI: 10.1136/eb-2015-102228]

34 **Rutter CM**, Gatsonis CA. A hierarchical regression approach to meta-analysis of diagnostic test accuracy evaluations. *Stat Med* 2001; **20**: 2865-2884 [PMID: 11568945 DOI: 10.1002/sim.942]

35 **Macaskill P**, Gatsonis C, Deeks J, Harbord R, Takwoingi Y. Analysing and Presenting Results: Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy. Version 1.0. The Cochrane Collaboration, 2010

36 **Takwoingi Y**, Guo B, Riley RD, Deeks JJ. Performance of methods for meta-analysis of diagnostic test accuracy with few studies or sparse data. *Stat Methods Med Res* 2017; **26**: 1896-1911 [PMID: 26116616 DOI: 10.1177/0962280215592269]

37 **Buczinski S**, Gicquel E, Fecteau G, Takwoingi Y, Chigerwe M, Vandeweerd JM. Systematic Review and Meta-Analysis of Diagnostic Accuracy of Serum Refractometry and Brix Refractometry for the Diagnosis of Inadequate Transfer of Passive Immunity in Calves. *J Vet Intern Med* 2018; **32**: 474-483 [PMID: 29210105 DOI: 10.1111/jvim.14893]

38 **Ng SC**, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, Panaccione R, Ghosh S, Wu JCY, Chan FKL, Sung JJY, Kaplan GG. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet* 2018; **390**: 2769-2778 [PMID: 29050646 DOI: 10.1016/S0140-6736(17)32448-0]

39 **Takwoingi Y**, Deeks J. MetaDAS: A SAS macro for meta-analysis of diagnostic accuracy studies: User guide, Version 1.3. 30 July 2010. Available from: http://srdta.cochrane.org/ Cited 17 March 2019

40 **The Nordic Cochrane Centre**. Review Manager; 2014. Cochrane Collab, 2014

41 **Bogdanos DP**, Roggenbuck D, Reinhold D, Wex T, Pavlidis P, von Arnim U, Malfertheiner P, Forbes A, Conrad K, Laass MW. Pancreatic-specific autoantibodies to glycoprotein 2 mirror disease location and behaviour in younger patients with Crohn's disease. *BMC Gastroenterol* 2012; **12**: 102 [PMID: 22866900 DOI: 10.1186/1471-230X-12-102]

42 **Bonaci-Nikolic B**, Spuran M, Andrejevic S, Nikolic M. Autoantibodies to GP2, the major zymogen granule membrane glycoprotein, in patients with gluten-sensitive enteropathy: a possible serological trap. *Clin Chim Acta* 2012; **413**: 822-823 [PMID: 22269156 DOI: 10.1016/j.cca.2012.01.005]

43 **Caneparo V**, Pastorelli L, Pisani LF, Bruni B, Prodam F, Boldorini R, Roggenbuck D, Vecchi M, Landolfo S, Gariglio M, De Andrea M. Distinct Anti-IFI16 and Anti-GP2 Antibodies in Inflammatory Bowel Disease and Their Variation with Infliximab Therapy. *Inflamm Bowel Dis* 2016; **22**: 2977-2987 [PMID: 27636380 DOI: 10.1097/MIB.0000000000000926]

44 **Cummings D**, Cruise M, Lopez R, Roggenbuck D, Jairath V, Wang Y, Shen B, Rieder F. Loss of tolerance to glycoprotein 2 isoforms 1 and 4 is associated with Crohn's disease of the pouch. *Aliment Pharmacol Ther* 2018; **48**: 1251-1259 [PMID: 30411391 DOI: 10.1111/apt.15034]

45 **Degenhardt F**, Dirmeier A, Lopez R, Lang S, Kunst C, Roggenbuck D, Reinhold D, Szymczak S, Rogler G, Klebl F, Franke A, Rieder F. Serologic Anti-GP2 Antibodies Are Associated with Genetic Polymorphisms, Fibrostenosis, and Need for Surgical Resection in Crohn's Disease. *Inflamm Bowel Dis* 2016; **22**: 2648-2657 [PMID: 27753692 DOI: 10.1097/MIB.0000000000000936]

46 **Gross S**, Bakker SF, van Bodegraven AA, van Hoogstraten IM, Gelderman KA, Bouma G, Mulder CJ, von Blomberg BM, Bontkes HJ. Increased IgA glycoprotein-2 specific antibody titres in refractory celiac disease. *J Gastrointestin Liver Dis* 2014; **23**: 127-133 [PMID: 24949603 DOI: 10.15403/jgld.2014.1121.232.sg1]

47 **Michaels MA**, Jendrek ST, Korf T, Nitzsche T, Teegen B, Komorowski L, Derer S, Schröder T, Baer F, Lehnert H, Büning J, Fellerman K, Sina C. Pancreatic Autoantibodies Against CUZD1 and GP2 Are Associated with Distinct Clinical Phenotypes of Crohn's Disease. *Inflamm Bowel Dis* 2015; **21**: 2864-2872 [PMID: 26273818 DOI: 10.1097/MIB.0000000000000564]

48 **Op De Beéck K**, Vermeire S, Rutgeerts P, Bossuyt X. Antibodies to GP2, the major zymogen granule membrane glycoprotein, in inflammatory bowel diseases. *Gut* 2012; **61**: 162-164; author reply 164-5 [PMID: 21193445 DOI: 10.1136/gut.2010.233148]

49 **Papp M**, Sipeki N, Tornai T, Altorjay I, Norman GL, Shums Z, Roggenbuck D, Fechner K, Stöcker W, Antal-Szalmas P, Veres G, Lakatos PL. Rediscovery of the Anti-Pancreatic Antibodies and Evaluation of their Prognostic Value in a Prospective Clinical Cohort of Crohn's Patients: The Importance of Specific Target Antigens [GP2 and CUZD1]. *J Crohns Colitis* 2015; **9**: 659-668 [PMID: 25968583 DOI: 10.1093/ecco-jcc/jjv087]

50 **Pavlidis P**, Romanidou O, Roggenbuck D, Mytilinaiou MG, Al-Sulttan F, Liaskos C, Smyk DS, Koutsoumpas AL, Rigopoulou EI, Conrad K, Forbes A, Bogdanos DP. Ileal inflammation may trigger the development of GP2-specific pancreatic autoantibodies in patients with Crohn's disease. *Clin Dev Immunol* 2012; **2012**: 640835 [PMID: 23118780 DOI: 10.1155/2012/640835]

51 **Pavlidis P**, Shums Z, Koutsoumpas AL, Milo J, Papp M, Umemura T, Lakatos PL, Smyk DS, Bogdanos DP, Forbes A, Norman GL. Diagnostic and clinical significance of Crohn's disease-specific anti-MZGP2 pancreatic antibodies by a novel ELISA. *Clin Chim Acta* 2015; **441**: 176-181 [PMID: 25512163 DOI: 10.1016/j.cca.2014.12.010]

52 **Pavlidis P**, Komorowski L, Teegen B, Liaskos C, Koutsoumpas AL, Smyk DS, Perricone C, Mytilinaiou MG, Stocker W, Forbes A, Bogdanos DP. Diagnostic and clinical significance of Crohn's disease-specific pancreatic anti-GP2 and anti-CUZD1 antibodies. *Clin Chem Lab Med* 2016; **54**: 249-256 [PMID: 26351932 DOI: 10.1515/cclm-2015-0376]

53 **Röber N**, Noß L, Goihl A, Reinhold D, Jahn J, de Laffolie J, Johannes W, Flemming GM, Roggenbuck D, Conrad K, Laass MW. Autoantibodies Against Glycoprotein 2 Isoforms in Pediatric Patients with Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2017; **23**: 1624-1636 [PMID: 28691939 DOI: 10.1097/MIB.0000000000001159]

54 **Roggenbuck D**, Reinhold D, Wex T, Goihl A, von Arnim U, Malfertheiner P, Büttner T, Porstmann T, Porstmann S, Liedvogel B, Bogdanos DP, Laass MW, Conrad K. Autoantibodies to GP2, the major zymogen granule membrane glycoprotein, are new markers in Crohn's disease. *Clin Chim Acta* 2011; **412**: 718-724 [PMID: 21195704 DOI: 10.1016/j.cca.2010.12.029]

55 **Roggenbuck D**, Hausdorf G, Martinez-Gamboa L, Reinhold D, Büttner T, Jungblut PR, Porstmann T, Laass MW, Henker J, Büning C, Feist E, Conrad K. Identification of GP2, the major zymogen granule membrane glycoprotein, as the autoantigen of pancreatic antibodies in Crohn's disease. *Gut* 2009; **58**: 1620-1628 [PMID: 19549613 DOI: 10.1136/gut.2008.162495]

56 **Roggenbuck D**, Vermeire S, Hoffman I, Reinhold D, Schierack P, Goihl A, von Arnim U, De Hertogh G, Polymeros D, Bogdanos DP, Bossuyt X. Evidence of Crohn's disease-related anti-glycoprotein 2 antibodies in patients with celiac disease. *Clin Chem Lab Med* 2015; **53**: 1349-1357 [PMID: 25411995 DOI: 10.1515/cclm-2014-0238]

57 **Zhang S**, Wu Z, Luo J, Ding X, Hu C, Li P, Deng C, Zhang F, Qian J, Li Y. Diagnostic Potential of Zymogen Granule Glycoprotein 2 Antibodies as Serologic Biomarkers in Chinese Patients With Crohn Disease. *Medicine (Baltimore)* 2015; **94**: e1654 [PMID: 26496271 DOI: 10.1097/MD.0000000000001654]

58 **Zhang S**, Luo J, Wu Z, Roggenbuck D, Schierack P, Reinhold D, Li J, Zeng X, Zhang F, Qian J, Li Y. Antibodies against glycoprotein 2 display diagnostic advantages over ASCA in distinguishing CD from intestinal tuberculosis and intestinal Behçet's disease. *Clin Transl Gastroenterol* 2018; **9**: e133 [PMID: 29446764 DOI: 10.1038/ctg.2018.1]

59 **Joossens S**, Vermeire S, Van Steen K, Godefridis G, Claessens G, Pierik M, Vlietinck R, Aerts R, Rutgeerts P, Bossuyt X. Pancreatic autoantibodies in inflammatory bowel disease. *Inflamm Bowel Dis* 2004; **10**: 771-777 [PMID: 15626896 DOI: 10.1097/00054725-200411000-00012]

60 **Stafylidou M**, Paschos P, Katsoula A, Malandris K, Ioakim K, Bekiari E, Haidich AB, Akriviadis E, Tsapas A. Performance of Baveno VI and Expanded Baveno VI Criteria for Excluding High-Risk Varices in Patients With Chronic Liver Diseases: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2019; **17**: 1744-1755.e11 [PMID: 31077823 DOI: 10.1016/j.cgh.2019.04.062]

61 **Lalkhen AG**, McCluskey A. Clinical tests: sensitivity and specificity. *Contin Educ Anaesth Crit Care Pain* 2008; **8**: 221–223 [DOI: 10.1093/bjaceaccp/mkn041]

62 **Power M**, Fell G, Wright M. Principles for high-quality, high-value testing. *Evid Based Med* 2013; **18**: 5-10 [PMID: 22740357 DOI: 10.1136/eb-2012-100645]

63 **Trevethan R**. Sensitivity, Specificity, and Predictive Values: Foundations, Pliabilities, and Pitfalls in Research and Practice. *Front Public Health* 2017; **5**: 307 [PMID: 29209603 DOI: 10.3389/fpubh.2017.00307]

64 **Fukuoka S**. Molecular cloning and sequences of cDNAs encoding alpha (large) and beta (small) isoforms of human pancreatic zymogen granule membrane-associated protein GP2. *Biochim Biophys Acta* 2000; **1491**: 376-380 [PMID: 10760606 DOI: 10.1016/s0167-4781(00)00057-9]

65 **Schmidt RL**, Factor RE. Understanding sources of bias in diagnostic accuracy studies. *Arch Pathol Lab Med* 2013; **137**: 558-565 [PMID: 23544945 DOI: 10.5858/arpa.2012-0198-RA]

66 **Al Fattani AG**, Aljoudi A. Sources of bias in diagnostic accuracy studies. *J Appl Hematol* 2015; **6**: 178 [DOI: 10.4103/1658-5127.171991]

67 Nature journals tighten rules on non-financial conflicts. *Nature* 2018; **554**: 6 [PMID: 29388964 DOI: 10.1038/d41586-018-01420-8]

68 **Tulleken C van**. Overdiagnosis and industry influence: how cow’s milk protein allergy is extending the reach of infant formula manufacturers. *BMJ* 2018; **363**: k5056 [DOI: 10.1136/bmj.k5056]

69 **Mintzes B**, Swandari S, Fabbri A, Grundy Q, Moynihan R, Bero L. Does industry-sponsored education foster overdiagnosis and overtreatment of depression, osteoporosis and over-active bladder syndrome? An Australian cohort study. *BMJ Open* 2018; **8**: e019027 [PMID: 29440213 DOI: 10.1136/bmjopen-2017-019027]

70 **Dakubo GD**.Cancer biomarkers in body fluids: principles. Ontario, Canada: Springer Nature; 2016; [DOI: 10.1007/978-3-319-01580-4]

71 **Thunnissen E**. How to Validate Predictive Immunohistochemistry Testing in Pathology? A Practical Approach Exploiting the Heterogeneity of Programmed Death Ligand-1 Present in Non-Small Cell Lung Cancer. *Arch Pathol Lab Med* 2019; **143**: 11-12 [PMID: 30307747 DOI: 10.5858/arpa.2018-0410-ED]

72 **Fitzgibbons PL**, Bradley LA, Fatheree LA, Alsabeh R, Fulton RS, Goldsmith JD, Haas TS, Karabakhtsian RG, Loykasek PA, Marolt MJ, Shen SS, Smith AT, Swanson PE; College of American Pathologists Pathology and Laboratory Quality Center. Principles of analytic validation of immunohistochemical assays: Guideline from the College of American Pathologists Pathology and Laboratory Quality Center. *Arch Pathol Lab Med* 2014; **138**: 1432-1443 [PMID: 24646069 DOI: 10.5858/arpa.2013-0610-CP]

73 **Magro F**, Lopes J, Borralho P, Lopes S, Coelho R, Cotter J, Castro FD, Sousa HT, Salgado M, Andrade P, Vieira AI, Figueiredo P, Caldeira P, Sousa A, Duarte MA, Ávila F, Silva J, Moleiro J, Mendes S, Giestas S, Ministro P, Sousa P, Gonçalves R, Gonçalves B, Oliveira A, Rosa I, Rodrigues M, Chagas C, Dias CC, Afonso J, Geboes K, Carneiro F; Portuguese IBD Study Group (GEDII). Comparison of different histological indexes in the assessment of UC activity and their accuracy regarding endoscopic outcomes and faecal calprotectin levels. *Gut* 2019; **68**: 594-603 [PMID: 29437913 DOI: 10.1136/gutjnl-2017-315545]

74 **Frykman PK**, Patel DC, Kim S, Cheng Z, Wester T, Nordenskjöld A, Kawaguchi A, Hui TT, Ehrlich PF, Granström AL, Benliyan F; HAEC Collaborative Research Group (HCRG). Inflammatory Bowel Disease Serological Immune Markers Anti-Saccharomyces cerevisiae Mannan Antibodies and Outer Membrane Porin C are Potential Biomarkers for Hirschsprung-associated Enterocolitis. *J Pediatr Gastroenterol Nutr* 2019; **69**: 176-181 [PMID: 30964819 DOI: 10.1097/MPG.0000000000002358]

75 **Freeman K**, Willis BH, Fraser H, Taylor-Phillips S, Clarke A. Faecal calprotectin to detect inflammatory bowel disease: a systematic review and exploratory meta-analysis of test accuracy. *BMJ Open* 2019; **9**: e027428 [PMID: 30852550 DOI: 10.1136/bmjopen-2018-027428]

76 **Jellema P**, van der Windt DA, Bruinvels DJ, Mallen CD, van Weyenberg SJ, Mulder CJ, de Vet HC. Value of symptoms and additional diagnostic tests for colorectal cancer in primary care: systematic review and meta-analysis. *BMJ* 2010; **340**: c1269 [PMID: 20360221 DOI: 10.1136/bmj.c1269]

77 **Cole SR**, Smith A, Wilson C, Turnbull D, Esterman A, Young GP. An advance notification letter increases participation in colorectal cancer screening. *J Med Screen* 2007; **14**: 73-75 [PMID: 17626705 DOI: 10.1258/096914107781261927]

78 **Gomollón F**, Dignass A, Annese V, Tilg H, Van Assche G, Lindsay JO, Peyrin-Biroulet L, Cullen GJ, Daperno M, Kucharzik T, Rieder F, Almer S, Armuzzi A, Harbord M, Langhorst J, Sans M, Chowers Y, Fiorino G, Juillerat P, Mantzaris GJ, Rizzello F, Vavricka S, Gionchetti P; ECCO. 3rd European Evidence-based Consensus on the Diagnosis and Management of Crohn's Disease 2016: Part 1: Diagnosis and Medical Management. *J Crohns Colitis* 2017; **11**: 3-25 [PMID: 27660341 DOI: 10.1093/ecco-jcc/jjw168]

79 **Kedia S**, Das P, Madhusudhan KS, Dattagupta S, Sharma R, Sahni P, Makharia G, Ahuja V. Differentiating Crohn's disease from intestinal tuberculosis. *World J Gastroenterol* 2019; **25**: 418-432 [PMID: 30700939 DOI: 10.3748/wjg.v25.i4.418]

80 **Zhang TY**, Lin Y, Fan R, Hu SR, Cheng MM, Zhang MC, Hong LW, Zhou XL, Wang ZT, Zhong J. Potential model for differential diagnosis between Crohn's disease and primary intestinal lymphoma. *World J Gastroenterol* 2016; **22**: 9411-9418 [PMID: 27895429 DOI: 10.3748/wjg.v22.i42.9411]

81 **Silverberg MS**, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, Caprilli R, Colombel JF, Gasche C, Geboes K, Jewell DP, Karban A, Loftus EV Jr, Peña AS, Riddell RH, Sachar DB, Schreiber S, Steinhart AH, Targan SR, Vermeire S, Warren BF. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol* 2005; **19** Suppl A: 5A-36A [PMID: 16151544 DOI: 10.1155/2005/269076]

82 **Levine A**, Griffiths A, Markowitz J, Wilson DC, Turner D, Russell RK, Fell J, Ruemmele FM, Walters T, Sherlock M, Dubinsky M, Hyams JS. Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. *Inflamm Bowel Dis* 2011; **17**: 1314-1321 [PMID: 21560194 DOI: 10.1002/ibd.21493]

83 **Komorowski L**, Teegen B, Probst C, Aulinger-Stöcker K, Sina C, Fellermann K, Stöcker W. Autoantibodies against exocrine pancreas in Crohn's disease are directed against two antigens: the glycoproteins CUZD1 and GP2. *J Crohns Colitis* 2013; **7**: 780-790 [PMID: 23140841 DOI: 10.1016/j.crohns.2012.10.011]

**Footnotes**

**Conflict-of-interest statement:** The authors deny any conflict of interest.

**PRISMA-DTA 2018 Checklist statement:** The authors have read the PRISMA-DTA 2018 Checklist, and the manuscript was prepared and revised according to the PRISMA-DTA 2018 Checklist.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**Manuscript source:**  Invited Manuscript

**Peer-review started:** November 1, 2019

**First decision:** November 22, 2019

**Article in press:** January 2, 2020

**Specialty type:** Gastroenterology and hepatology

**Country of origin:** Greece

**Peer-review report classification**

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0

**P-Reviewer:** Mattar MC, Sachar D **S-Editor:** Tang JZ **L-Editor:** A **E-Editor:** Ma YJ

**Table 1** **Search strategy for PubMed and Cochrane-CENTRAL**

|  |  |
| --- | --- |
| **Database** | **Key words** |
| PubMed |  |
| No. 1 | anti-glycoprotein 2 antibody |
| No. 2 | "anti-glycoprotein 2 antibody" |
| No. 3 | anti-glycoprotein 2 antibody [Text Words] |
| No. 4 | autoantibodies to glycoprotein 2 |
| No. 5 | "autoantibodies to glycoprotein 2" |
| No. 6 | autoantibodies to glycoprotein 2 [Text Words] |
| No. 7 | "glycoprotein 2 autoantibodies" |
| No. 8 | glycoprotein 2 autoantibodies [Text Words] |
| No. 9 | autoantibodies (as a MeSH term) |
| No. 10 | OR (Νο. 1 – No. 9) |
| No. 11 | Crohn’s disease |
| No. 12 | Crohn's disease (as a MeSH term) |
| No. 13 | OR (No. 11, No. 12) |
| No. 14 | AND (No. 10, No. 13) |
| Cochrane-CENTRAL |  |
| No. 1 | anti-glycoprotein 2 antibody |
| No. 2 | autoantibodies to glycoprotein 2 |
| No. 3 | autoantibodies (as a MeSH term) |
| No. 4 | OR (No. 1 – No. 3) |
| No. 5 | Crohn’s disease |
| No. 6 | Crohn's disease (as a MeSH term) |
| No. 7 | OR (No. 5, No. 6) |
| No. 8 | AND (No. 4, No. 7) |

No. means order of the keywords entered on PubMed and CENTRAL. OR and AND are Boolean operators used between keywords.

**Table 2** **Characteristics of the included studies evaluating the diagnostic accuracy of glycoprotein 2 antibodies in Crohn’s disease**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Ref.** | **Country** | **Multicenter** | **Recruitment** | | | **CD diagnosis and classification** | | **Assays characteristics** | | | | | **Sample characteristics** | | | | | **Funding** |
| **Year** | **Site** | **Consecutive** | **CD diagnosis** | **Montreal[81]** | **Assay** | **Kit** | **anti-GP2 form** | **Blind assay** | **Positivity cut-off U/dL** | **N9** | **Unrelated sample** | **Children included** | **Sex (% female)** | **Age (yr)5** |
| Bogdanos *et al*[41] | Germany;  United Kingdom | √ | NR | Otto-von-Guericke University; UCL Hospital; and Children’s hospital Technical University Dresden | NR | Standard clinical, radiological, endoscopical and histological criteria[8,9] | √ | ELISA | GA (Dahlewitz  Berlin Germany) | Total | No | IgA 20; IgG 20 | CD *n* = 169; UC *n* = 102; HC *n* = 225 | NR | √ | CD 60.3%; UC 55.9% | CD 36 (8–87)1; UC 47 (17–92)1 | Higher Education Funding Council of England; Biomedical Research Centre, United Kingdom NIHR; and Brandenburg Ministry of Economics; and EU |
| Bonaci-Nikolic *et al*[42] | Serbia | NR | NR | Clinical Center of Serbia | NR | Clinical, endoscopic, histologic, lab findings[21] | - | ELISA | GA (Dahlewitz/  Berlin Germany) | Total | No | IgA 20; IgG 24 | CD *n* = 33; UC *n* = 23; GSE *n* = 21; HC *n* = 13 | NR | - | CD 42.4%; UC 56.5%; GSE 76.2%; HC 46.1% | CD 35 (19–63)4;  UC 34 (24–57)4; GSE 31 (19–57)4; HC 41 (22–55) | None declared |
| Caneparo *et al*[43] | Italy | - | 2008–2014 | Policlinico San Donato | NR | Clinical, endoscopic and histological criteria | - | ELISA | GA (Dahlewitz/  Berlin Germany) | Total | √ | IgA 10; IgG 15 | CD n=48  UC n=26  HC n=182 | NR | √ | CD 47.9%; UC 19.2% | CD 41 (16–65)4; UC 39 (17–62)4 | Regione Piemonte and Letizia Castelli Schubert Foundation |
| Cummings *et al*[44] | United Kingdom | - | 2009 –2010 | Cleveland Clinic | √ | Clinical, endoscopic, radiographic, histopathological criteria[22] | - | ELISA | GA (Dahlewitz/  Berlin Germany) | Isoforms  GP21 and GP24 | √ | IgA GP21 14; IgA GP24 3.7; IgG GP21 18; IgG GP24 15 | UC *n* = 117 | √ | - | UC 44.4% | UC 44.3 ± 13.7 | Obtained but not disclosed |
| Degenhardt *et al*[45] | Germany | - | 2000–2006 | University Medical Center Regensburg | NR | European Crohn and Colitis Organization criteria[23] | √ | ELISA | GA (Dahlewitz/  Berlin Germany) | Isoform alpha and betta | √ |  | CD *n* =303; UC *n* =108; OGD *n* = 72; OPC *n* = 206 | NR | √ | CD 52.8%; UC 39.8%; ODG 36.1% | CD 36.1 ± 12.5; UC 40.2 ± 12.8; OGD 60.3 ± 13.8 | Bundesministerium fur Bildung & Forschung and Kompetenznetz chronisch entzundliche Darmerkrankungen |
| Gross *et al*[46] | Netherlands | NR | NR | VU University Medical Center Amsterdam | - | Lennard-Jones criteria[24] | - | ELISA | GA (Dahlewitz/  Berlin Germany) | Total | No | IgA 20; IgG 20 | CD *n* = 38; UC *n* = 40; CeD *n* = 45; GFD *n* = 34; RCD *n* = 15 | NR | - | CD 71.1%; UC 52.5% | CD 36.4 ± 11.8; UC 36.5 ± 9.6 | CD consortium |
| Michaels *et al*[47] | Germany | - | 2005–2013 | University Hospital Schleswig-Holstein | - | Typical clinical, endoscopical, histological and/or radiological findings of CD/UC | √ | IIF | Euroimmun Germany | Total | NR |  | CD *n* = 224; UC *n* = 136 | NR | NR | CD 64.3%; UC 54.4% | CD 392; UC 422 | Else-Kröner-Fresenius-Stiftung |
| Op De Beéck *et al*[48] | Belgium | - | NR | University Hospital Gasthuisberg, Leuven | NR | Lennard-Jones criteria[24] | - | ELISA | GA (Dahlewitz/  Berlin Germany) | Total | NR | IgA 15; IgG 15 | CD *n* = 164; UC *n* = 118; OGD *n* = 75 | NR | √ | CD 58.6%6; UC 41.7%6[59]; ODG NR | CD 42 (17–80)1,6; UC 43 (19–78)1,6[59]; ODG NR | Fund for Scientific Research Flanders and GA GmbH |
| Papp *et al*[49] | Hungary | - | 2005–2010 | Institute of Internal Medicine, University of Debrecen | √ | Lennard-Jones criteria[24] | √ | ELISA | GA (Dahlewitz/  Berlin Germany) | Total | √ | IgA 20; IgG 20 | CD *n* = 271; UC *n* = 187; HC *n* = 100 | NR | - | CD 61.5%; UC 54% | CD 25 (19–33); UC 33 (23–43) | Janos Bolyai Scholarship; Debrecen University; and IOIBD Research |
| Pavlidis *et al*[50] | United Kingdom | - | NR | UCL Hospital | NR | Lennard-Jones criteria[24] | √ | ELISA | GA (Dahlewitz/  Berlin Germany) | Total | NR | IgG 20 | CD *n* = 225; UC *n* = 225 | NR | - | CD 56.4%; UC 49.7% | CD 36 ± 14.3; UC 51 ± 15.7 | NIHR; Higher Education Funding Council for England; EASL; and INOVA Diagnostics |
| Pavlidis *et al*[51] | United Kingdom | - | NR | UCL Hospital | √ | Lennard-Jones criteria[24] | √ | ELISA | Inova Diagnostics (Research use only) | Total | √ | IgA 20; IgG 25 | CD *n* = 323; UC *n* = 294; OPC *n* = 112; HC *n* = 103 | NR | - | CD 54%; UC 47.9% | CD 40 ± 14.3; UC 48.7 ± 15.7 | INOVA Diagnostics |
| Pavlidis *et al*[52] | United Kingdom | - | NR | UCL Hospital | NR | Lennard-Jones criteria[24] | √ | IIF | N/A8 | Total | NR |  | CD *n* = 212; UC *n* = 249 | NR | - | CD 42.4%; UC 51.4% | CD 42.4 (30–49); UC 51.4 (37–61) | Euroimmun |
| Röber *et al*[53] | Germany | √ | 1994-2014 | Three children’ s University hospitals (Dresden, Leipzig, Giessen) | NR | Porto criteria[25] | Paris  [82] | ELISA | GP21, GP23: (AMS Biotechnology, Abingdon, United Kingdom); GP22: (CCS GmbH, Hamburg, Germany);  GP24: (Thermo Sci, Braunschweig, Germany) | Isoforms 1, 2, 3, 4 | NR | IgA GP21 7.02; IgA GP22 7.33; IgA GP23 4.37; IgA GP24 9.01; IgG GP21 33.38; IgG GP22 71.75; IgG GP23 15.89; IgG GP24 23.22 | CD *n* = 164; UC *n* = 114; GE *n* = 27; ENDO *n* =56; HC *n* =218 | NR | √ | CD 39.6%; UC 54.3%; GE 52%; ENDO 50% | CD 13 (10–15); UC 14 (11–15); GE 2 (1–5); ENDO 13 (7–16) | None declared |
| Roggenbuck *et al*[56] | Germany; Greece; Belgium | √ | NR | Attikon Hospital, UoA; Otto-von-Guericke University; and University Hospital Leuven | NR | NR. Communication with an author, confirmed the Lennard-Jones criteria[24] | - | ELISA | GA (Dahlewitz/  Berlin Germany) | Total | NR |  | CD *n* = 73; CeD *n* = 79; HC *n* = 90 | NR | √7 | CD 52%; CeD 69.6% | CD 36.5 (30–43)3; CeD 24 (12–42)3 | None declared |
| Roggenbuck *et al*[55] | Germany | - | NR | Charité Berlin | NR | Lennard-Jones criteria[24] | - | ELISA | GA (Dahlewitz/  Berlin Germany) | Total | NR | IgA 20; IgG 20 | CD *n* = 73; UC *n* = 49; HC *n* = 63 | NR | - | CD 57.5%; UC 59.1% | CD 41 (20–72)1; UC 40 (21–71)1 | Brandenburg Ministry of Economics and EU |
| Roggenbuck *et al*[54] | Germany | NR | NR | NR | √ | Lennard-Jones criteria[24] | NR | ELISA | GA (Dahlewitz/  Berlin Germany) | Total | NR | IgA 20; IgG 20 | CD *n* = 178; UC *n* = 100; HC *n* = 162 | NR | - | CD 60.7%; UC 54% | CD 39 (18–87)1; UC 42 (18–71)1 | Brandenburg Ministry of Economics and EU |
| Zhang *et al*[57] | China | - | NR | Peking Union Medical College Hospital | NR | Lennard-Jones criteria[24] | √ | ELISA | GA (Dahlewitz/  Berlin Germany) | Total | NR | IgA 20; IgG 20 | CD *n* = 35; UC *n* = 35; OGD *n* = 13; HC *n* = 8 | NR | √ | CD 17%; UC 38%; ODG NR | CD: 17 (13–69)1; UC: 38 (18–75)1; ODG NR | NNSFC |
| Zhang *et al*[58] | China | - | NR | Peking Union Medical College Hospital | √ | Lennard-Jones criteria[24] | √ | ELISA | GA (Dahlewitz/  Berlin Germany) | Total | NR | IgA 10; IgG 15 | CD *n* = 171; UC *n* = 208; BD *n* = 71; ITB *n* = 57; HC *n* = 70 | NR | √ | CD 33%; UC 43%; BD 38%; ITB 43% | CD 33 (10–85)1; UC 43 (12–77)1; BD 38 (10–73)1; ITB 43 (14–76)1 | NNSFC; Chinese Academy of Medical Sciences; and Chinese Key Research & Development Program |

1Median (minimum–maximum). 2median. 3Mean (IQR1–IQR3). 4Mean (minimum–maximum). 5mean ± standard deviation, or median, IQR. 6Not all participants from the Joossens[59] study were included. 7Not among participants with Crohn’s disease. 8Using as substrates IIF chip slides containing sections of unfixed pancreas, recombinantly transformed HEK293 cells-overexpressing GP2[83]. 9Healthy controls were not included in the sensitivity and specificity analyses herein. Anti-GP2: Glycoprotein 2 antibodies; BD: Behçet's disease; CD: Crohn’s disease; CeD: Celiac Disease; EASL: European association for the study of liver; ENDO: Nonspecific gastrointestinal symptoms; ELISA: Enzyme-linked immunosorbent assay; EU: European Union; GA: Generic assays; GE: Acute gastroenteritis; GFD: CeD on gluten-free diet; GP2: Glycoprotein 2; GSE: Gluten-sensitive enteropathy; HC: Healthy controls; IBD: Inflammatory bowel diseases; IIF: Indirect immune-fluorescence; ITB: Intestinal tuberculosis; N/A: Not applicable; NIHR: National Institute for Health Research; NNSFC: National Natural Science Foundation of China; NR: Not reported; OGD: Other gastrointestinal disease; OPC: Other pathological conditions; RCD: Refractory Crohn’s disease; UC: Ulcerative Colitis; UCL: University College London; UoA: University of Athens.

**Table 3 Investigation of heterogeneity (meta-regression)**

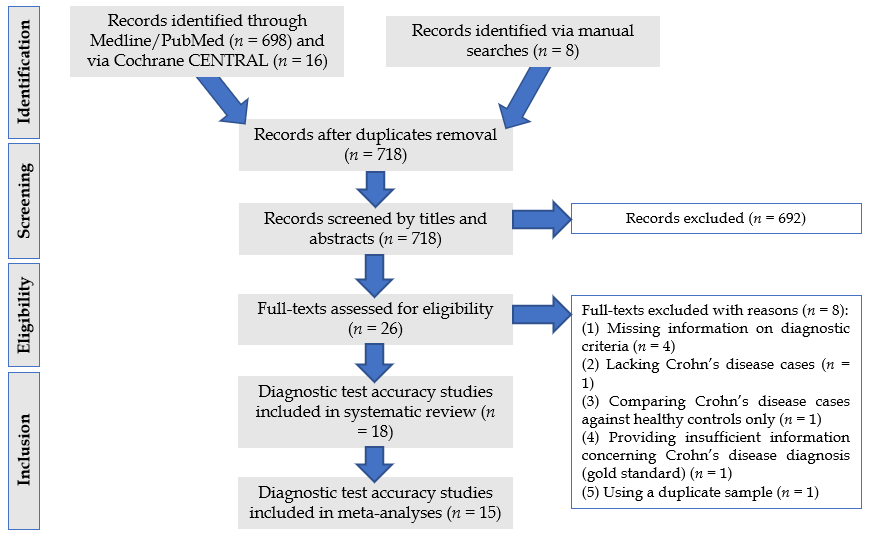
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Covariate** |  | **Population** | **Ig Type** | **Number of studies** | **Relative diagnostic**  **Odds ratio (95%CI)** |
| Funding type | State *vs* Other | CD *vs* All | IgG | 6 *vs* 9 | 1.91 (0.87–4.21) |
|  |  |  | IgA | 5 *vs* 9 | 1.08 (0.38–3.06) |
|  |  | CD *vs* UC | IgG | 5 *vs* 8 | 1.21 (0.81–1.80) |
| COI | Industry-related COI *vs* no apparent industry-related COI | CD *vs* All | IgG | 7 *vs* 8 | 0.73 (0.32–1.66) |
|  |  | IgA | 6 *vs* 8 | 0.53 (0.21–1.30) |
|  |  | CD *vs* UC | IgG | 9 *vs* 4 | 0.48 (0.19–1.20) |
| Method | ELISA *vs* IFF | CD *vs* All | IgG | 13 *vs* 2 | 0.84 (0.38–1.85) |
|  |  |  | IgA | 12 *vs* 2 | 4.25 (1.26–14.37) |
|  |  | CD *vs* UC | IgG | 11 *vs* 2 | 1.60 (0.40–6.54) |
| Blind assay | No/not stated *vs* Yes | CD *vs* All | IgG | 12 *vs* 3 | 3.28 (1.33–8.09) |
|  |  |  | IgA | 11 *vs* 3 | 1.77 (0.63–5.00) |
|  |  | CD *vs* UC | IgG | 10 *vs* 3 | 1.15 (0.32–4.15) |
| Consecutive sampling | No/not stated *vs* Yes | CD *vs* All | IgG | 11 *vs* 4 | 1.47 (0.65–3.32) |
|  |  |  | IgA | 10 *vs* 4 | 1.31 (0.53–3.21) |
|  |  | CD *vs* UC | IgG | 9 *vs* 4 | 1.88 (0.65–5.38) |
| Kit manufacturer | GA *vs* All other | CD *vs* All | IgG | 12 *vs* 3 | 1.04 (0.51–2.11) |
|  |  |  | IgA | 11 *vs* 3 | 1.28 (0.80–2.03) |
|  |  | CD *vs* UC | IgG | 10 *vs* 3 | 1.47 (0.48–4.48) |
| Female participants | ≥ 50% *vs* < 50% | CD *vs* All | IgG | 11 *vs* 4 | 1.24 (0.79–1.94) |
|  |  |  | IgA | 10 *vs* 4 | 0.75 (0.30–1.93) |
|  |  | CD *vs* UC | IgG | 10 *vs* 3 | 1.15 (0.54–2.45) |

CD: Crohn’s disease; CI: Confidence interval; COI: Conflict of interest; GA: Generic assays; ELISA: Enzyme-linked immunosorbent assay; IFF: Indirect immune-fluorescence; Ig: Immunoglobulin; UC: Ulcerative Colitis.

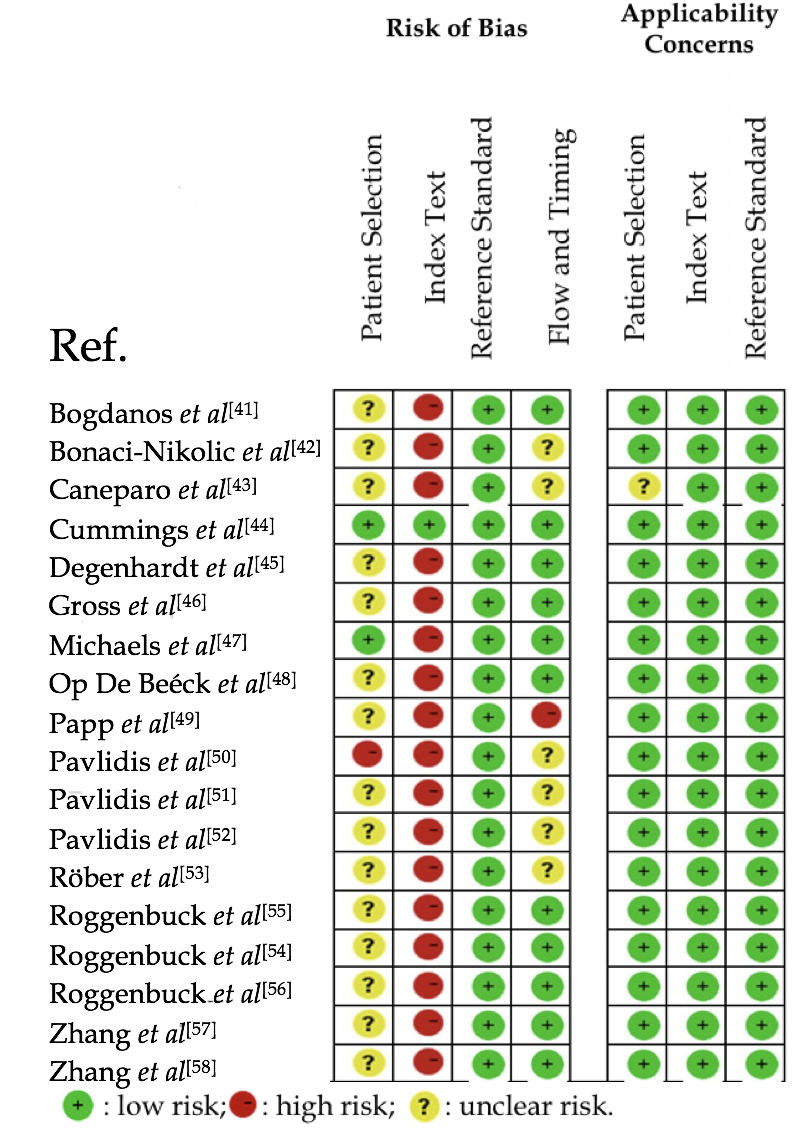
**Table 4 Summary of findings table based on a hypothetical scenario[38] of applying glycoprotein 2 antibodies tests on a cohort of 10000 patients**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Analysis** | **Diagnostic**  **cut-off (U/dL)** | **TP** | **(Range)** | **FP** | **(Range)** | **TN** | **(Range)** | **FN** | **(Range)** |
| CD *vs* All symptomatic patients (IgG) | Mixed | 9 | (6–21) | 698 |  | 9270 |  | 23 | (21–26) |
| 20 | 7 | (5–10) | 698 | (498–897) | 9270 | (9071–9470) | 25 | (22–27) |
| 15 | 9 | (5–14) | 797 | (399–1,595) | 9171 | (8373–9569) | 23 | (18–27) |
| CD *vs* UC (IgG) | Mixed | 10 | (8–12) | 698 |  | 9270 |  | 22 | (20–24) |
| 20 | 8 | (5–11) | 698 | (399–997) | 9270 | (8971–9569) | 24 | (21–27) |
| 15 | 9 | (5–14) | 997 | (598–1595) | 8971 | (8373–9370) | 23 | (18–27) |
| CD *vs* All symptomatic patients (IgA) | Mixed | 5 | (4–6) | 299 |  | 9,669 |  | 27 | (26–28) |
| 20 | 5 | (3–8) | 399 | (100–1396) | 9569 | (8572–9868) | 27 | (24–29) |
| CD *vs* UC (IgA) | Mixed | 4 | (1–6) | 199 |  | 9769 |  | 28 | (26–31) |
| 20 | 5 | (3–7) | 199 | (100–399) | 9769 | (9569–9868) | 27 | (25–27) |
| CD *vs* All symptomatic patients (IgA and/or IgG) | Mixed | 6 | (3–9) | 299 |  | 9669 |  | 26 | (20–23) |
| 20 | 7 | (4–12) | 698 | (199–1994) | 9270 | (7974–9769) | 25 | (20–28) |
| CD *vs* UC (IgA and/or IgG) | Mixed | 6 | (1–11) | 299 |  | 9669 |  | 26 | (21–31) |

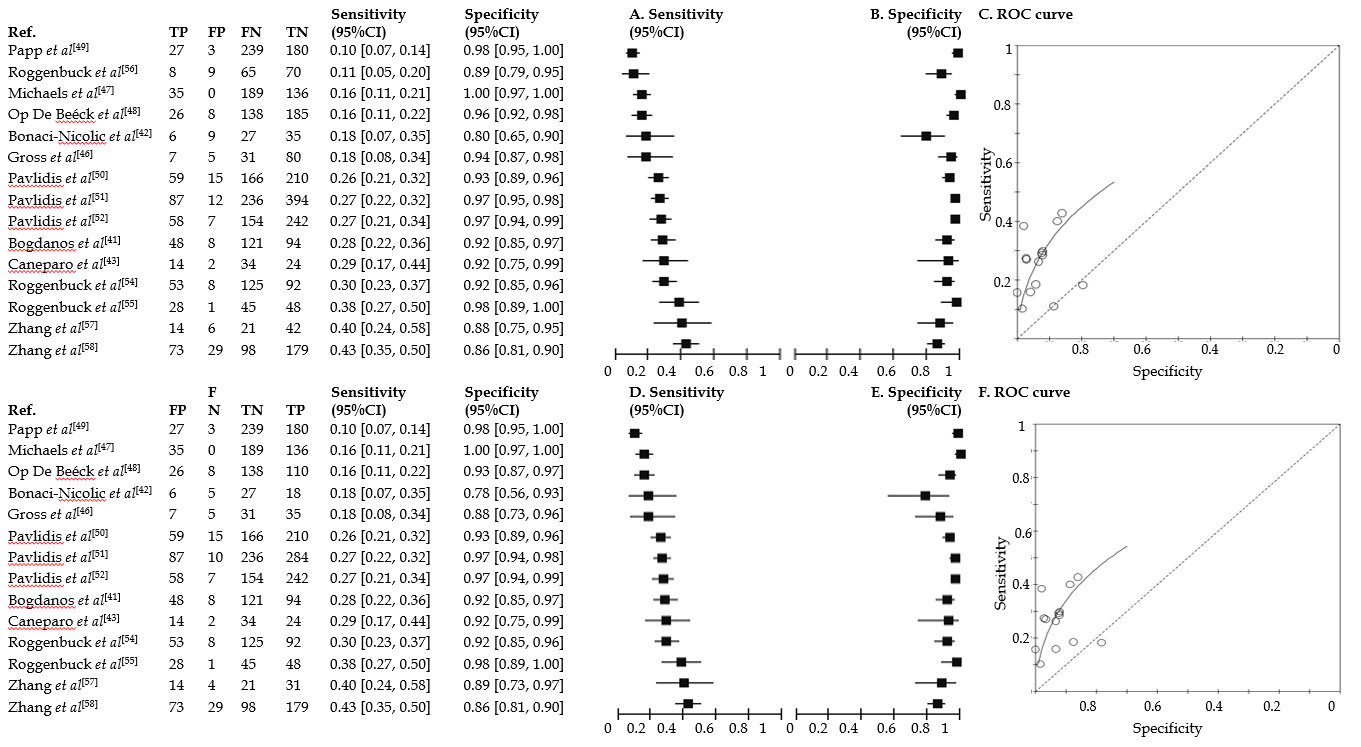
Mixed diagnostic cut-off includes all cut-offs used as well as studies with unreported cut-offs. A prevalence of 322 per 100000 results in 32 patients with Crohn’s disease in this cohort. FN: False negatives; FP: False positives; TN: True negatives; TP: True positives; UC: Ulcerative colitis; Ig: Immunoglobulin.



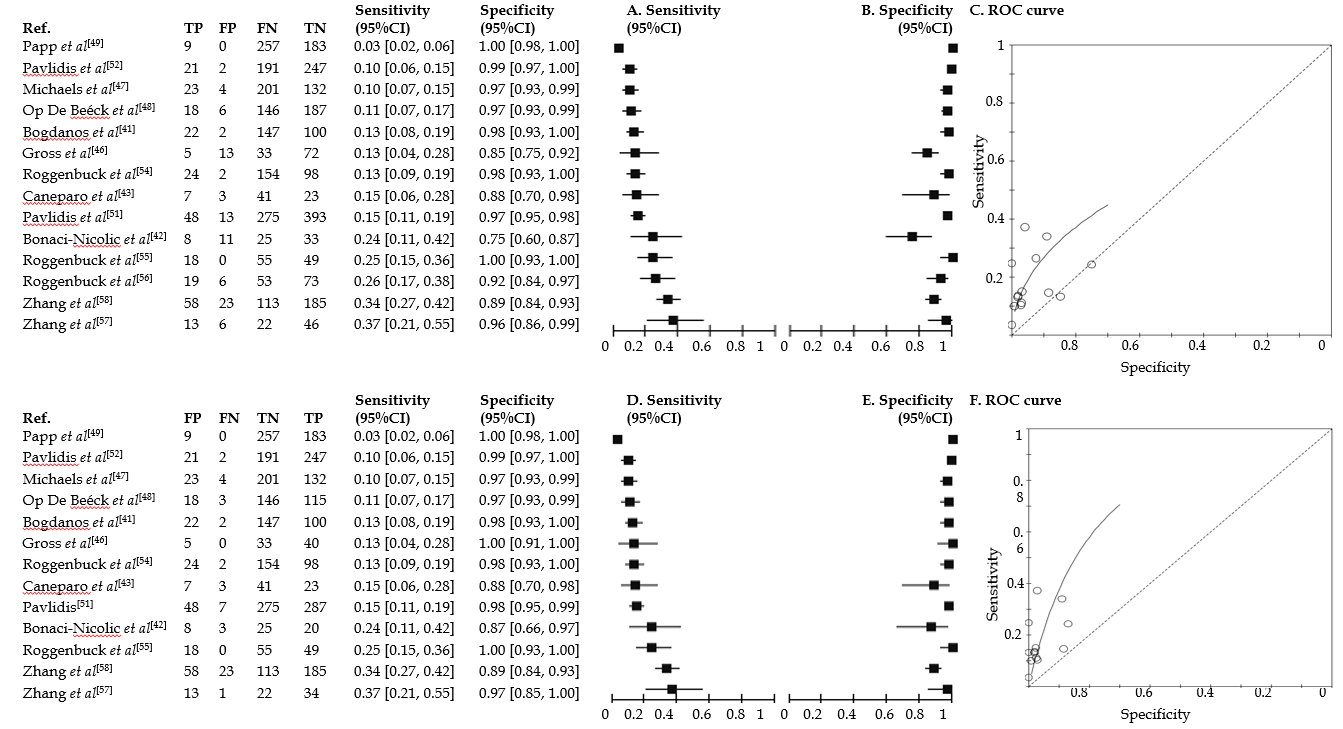
**Figure 1 Flowchart of the diagnostic test accuracy studies selection.**

****

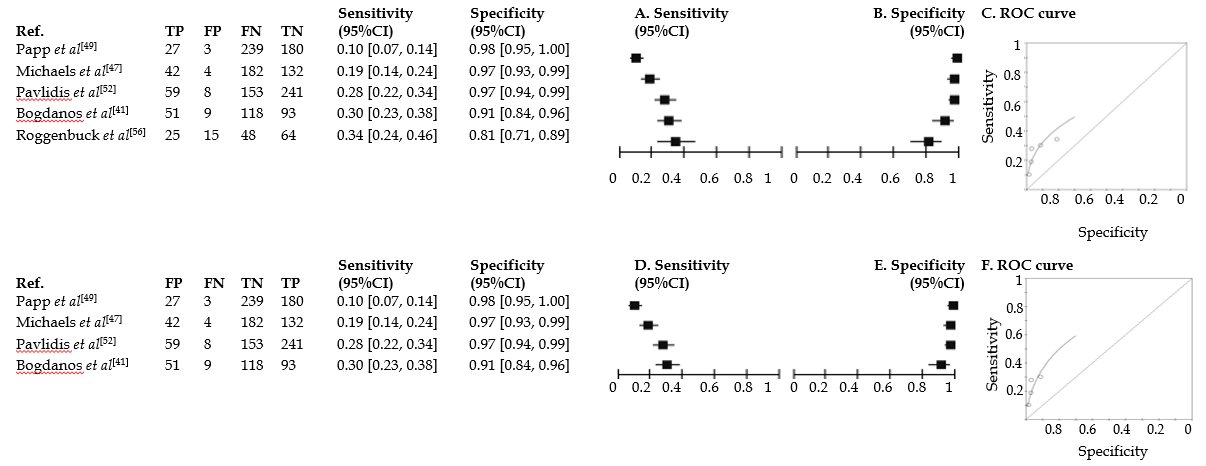
**Figure 2 Quality assessment of the included studies based on the quality assessment of diagnostic accuracy studies-2 tool[31].**



**Figure 3** **Pooled forest plots.** A-C: Pooled forest plots for sensitivity and specificity, and the summary receiver operating characteristic curve of anti-GP2 antibody (IgG positive) for Crohn’s Disease against all patients with relevant symptoms. D-F: Pooled forest plots for sensitivity and specificity, and the summary receiver operating characteristic curve of anti-GP2 antibody (IgG positive) for patients with Crohn’s Disease against patients with ulcerative colitis. FN: False negative; FP: False positive; ROC: Receiver operating characteristic; TN: True negative; TP: True positive; CI: Confidence interval.



**Figure 4 Pooled forest plots.** A-C: Pooled forest plots for sensitivity and specificity, and the summary receiver operating characteristic curves of anti-GP2 antibody (IgA positive) for Crohn’s Disease against all patients with relevant symptoms; D-F: Pooled forest plots for sensitivity and specificity, and the summary receiver operating characteristic curves of anti-GP2 antibody (IgA positive) for patients with Crohn’s Disease against patients with Ulcerative colitis. FN: False negative; FP: False positive; ROC: Receiver operating characteristic; TN: True negative; TP: True positive; CI: Confidence interval.



**Figure 5** **Pooled forest plots.** A-C: Pooled forest plots for sensitivity and specificity, and the summary receiver operating characteristic curves of anti-GP2 antibody (IgA and/or IgG positive) for Crohn’s Disease against all patients with relevant symptoms; D-F: Pooled forest plots for sensitivity and specificity, and the summary receiver operating characteristic curves of anti-GP2 antibody (IgA and/or IgG positive) for patients with Crohn’s Disease against patients with Ulcerative colitis. FN: False negative; FP: False positive; ROC: Receiver operating characteristic; TN: True negative; TP: True positive; CI: Confidence interval.