ORIGINAL ARTICLE

Serum Anti-glycan Antibody Biomarkers for Inflammatory Bowel Disease Diagnosis and Progression: A Systematic Review and Meta-analysis

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Background: Anti-glycan antibody serologic markers may serve as a useful adjunct in the diagnosis/prognosis of inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC). This meta-analysis/systemic review aimed to evaluate the diagnostic value, as well as the association of anti-glycan biomarkers with IBD susceptible gene variants, disease complications, and the need for surgery in IBD.

Methods: The diagnostic odds ratio (DOR), 95% confidence interval (CI), and sensitivity/specificity were used to compare the diagnostic value of individual and combinations of anti-glycan markers and their association with disease course (complication and/or need for surgery).

Results: Fourteen studies were included in the systemic review and nine in the meta-analysis. Individually, anti-*Saccharomyces cervisiae* antibodies (ASCA) had the highest DOR for differentiating IBD from healthy (DOR 21.1; 1.8–247.3; two studies), and CD from UC (DOR 10.2; CI 7.7–13.7; seven studies). For combination of $\geq$2 markers, the DOR was 2.8 (CI 2.2–3.6; two studies) for CD-related surgery, higher than any individual marker, while the DOR for differentiating CD from UC was 10.2 (CI 5.6–18.5; three studies) and for complication was 2.8 (CI 2.2–3.7; two studies), similar to individual markers.

Conclusions: ASCA had the highest diagnostic value among individual anti-glycan markers. While anti-chitobioside carbohydrate antibody (ACCA) had the highest association with complications, ASCA and ACCA associated equally with the need for surgery. Although in most individual studies the combination of $\geq$2 markers had a better diagnostic value as well as higher association with complications and need for surgery, we found the combination performing slightly better than any individual marker in our meta-analysis.

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Key Words: inflammatory bowel disease, Crohn's disease (CD), ulcerative colitis (UC), anti-glycan, serological biomarkers, meta-analysis, systemic review, disease complication, surgery for IBD

Inflammatory bowel disease (IBD) is thought to be the result of an aberrant immunological response to commensal microbes in genetically susceptible individuals.[1–4] Serum antibodies against microbes or self-antigens have been used as markers for the disease phenotype and disease course in Crohn's disease (CD) and ulcerative colitis (UC).[5–11] Although the mechanism is unclear, these serological biomarkers may be the consequence of injury to the gut and/or increased permeability to the luminal microbes or other agents. Anti-*Saccharomyces cerevisiae* antibodies (ASCA) and perinuclear antineutrophil cytoplasmic antibodies (pANCA) were the first extensively characterized serological IBD markers.[12,13] Additionally, there are other serum biomarkers for diagnostic use or for assessing their association with disease complication in IBD, including antibodies against outer membrane porin C (anti-OmpC), *Pseudomonas fluorescens* bacterial sequence I2 (anti-I2), and bacterial flagellin (anti-CBir 1).[6,14–16]

Glycans are the predominant cell wall surface components in many saprophytic and pathogenic fungi, yeast, and bacteria, as well as protozoa and viruses. Antibodies to these glycans have been shown to be effective markers for the disease phenotype, with potential predictive value for disease course and treatment stratification of IBD.[17,18] In addition to ASCA, the anti-glycan antibodies also include AMCA (anti-mannobioside carbohydrate antibody), ALCA

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(anti-laminaribioside carbohydrate antibody), ACCA (anti-chitobioside carbohydrate antibody), Anti-L (anti-laminarin), and Anti-C (anti-chitin).[17,18] Several independent studies have reported on the diagnostic ability of these markers and their association with disease complication (see review[33]), but the results and conclusions vary between studies. Therefore, a meta-analysis of these data is necessary.

We aimed to perform a systematic review and meta-analysis of the diagnostic ability of the anti-glycan antibodies to differentiate IBD from non-IBD and CD from UC, as well as their association with disease complications and/or the need for surgery in IBD.

MATERIALS AND METHODS

Search Strategy

The most recent search of Medline was performed in May 2011. The search strategy was: ("Inflammatory Bowel Disease" or "Crohn" or "Ulcerative Colitis") and Glycan and Antibody. No language restrictions were made, but we did not identify any non-English studies that met the inclusion criteria based on the titles and abstracts.

Inclusion and Exclusion Criteria

Included studies compared at least two of the six anti-glycan antibody markers (ASCA [or gASCA], AMCA, ALCA, ACCA, Anti-L, and Anti-C) in human subjects with at least one of the following outcomes: differentiating IBD from non-IBD; CD from UC; IBD-related complication; or need for IBD-related surgery. gASCA, so termed in the anti-glycan panel from Glycominds (Simi Valley, CA), is equivalent to ASCA termed in other assays made by other commercial sources. We excluded reviews, case reports, and editorials.

Review Processes and Data Abstraction

Title, abstract, and full article selection were performed independently by two reviewers (A.K., S.H.) with conflicts resolved by consensus adjudication.

Outcomes

The primary outcomes of interest were to differentiate IBD from non-IBD and CD from UC. Secondary outcome of interest was to analyze and compare the association of these markers with disease course including complications and/or need for surgery in IBD.

Statistical Analysis

Pooled sensitivity and specificity were calculated using a DerSimonian and Laird random-effects model and summarized with the diagnostic odds ratio (DOR), which compares the odds of being correctly classified (true positive or negative) to being incorrectly classified (false positive or negative). The DOR was calculated for individual anti-glycan markers as well as combinations of markers. The only combination possible for meta-analysis was ≥ 2 markers compared to ≤ 1 marker. I-squared was used to assess the statistical heterogeneity with values of 50% and greater indicating significant heterogeneity. We used MetaAnalyst, Beta 3.1 software[19] for Windows and Stata 11.0 for all analyses.[20]

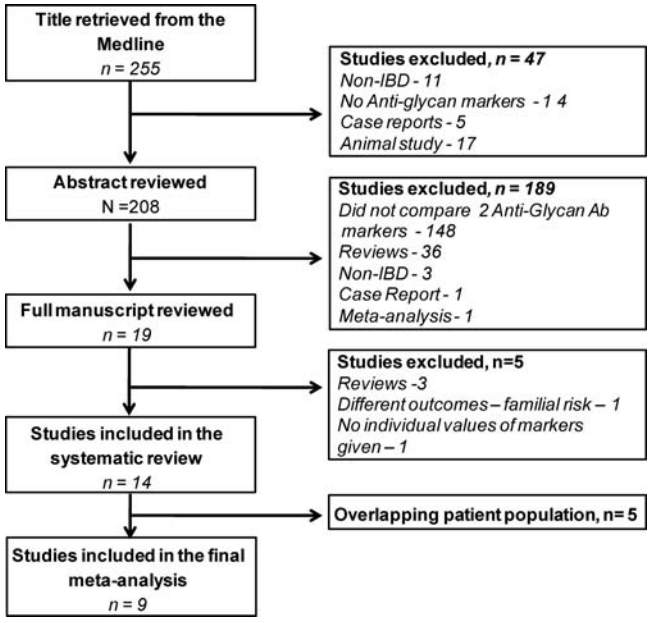

FIGURE 1. Flow chart for the selection of the studies in the systematic review and meta-analysis.

RESULTS AND DISCUSSION

Study Characteristics

The studies included at each level of review and the reasons for exclusions are illustrated in Figure 1. Fourteen studies were included in the systematic review (Table 1). Of these included studies, only nine were included in the meta-analysis due to possible overlap of patient populations (Table 1 highlights the excluded studies, and Table 2 lists the studies included in the meta-analysis). We contacted the corresponding authors of the five studies,[21–25] and received one reply confirming that the study had an overlapping patient population. Therefore, we included the study with the largest sample size of those studies. All 14 included studies were retrospective and occurred at referral centers (Table 1). Twelve studies were conducted in Europe, one in Israel, and one in Canada. Only two of the studies[26,27] included in the meta-analysis reported the sensitivities and specificities of anti-C and anti-L.

The pooled analyses of the nine studies included in the meta-analysis are summarized in Table 3. We also compared the 9 versus 14 studies, and found the DORs of ASCA for all the different diagnostic differentiation outcomes were higher when all 14 studies (Supporting Table 1) were analyzed together, as compared to 9 (Table 3). The DORs for surgery, complications, and combination of

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TABLE 1. Study Population Characteristics; Serum Markers Measured, Outcomes Reported in Studies Included in the Systematic Review

	Source population	CD *n*	UC *n*	IBD *n*	OGD *n*	Healthy *n*	Serum Markers Measured						Clinical Outcomes Reported			
							ASCA	AMCA	ALCA	ACCA	Anti-L	Anti-C	Fistula/ Stricture	Perianal Disease	Disease Location	Surgery
Koutroubakis 2011 Greece	Cases: Consecutive IBD patients from gastroenterology departments of 2 hospitals Healthy Controls: Blood donors, hospital employees and visitors of Obstetrics-Gynecology and Orthopedics wards matched to cases on age and sex, but with no family history of IBD. OGD: Ischemic colitis, infectious colitis, and diverticulitis cases. Unclear recruitment source. Time-period: not specified	106	85	191	29	96	√	√	√	√	—	—	√	—	√	√
Malickova 2010 Czech Republic	Cases: Serum samples were derived from the IBD Serum Bank of the Institute of Clinical Biochemistry and Laboratory Diagnostics, General University Hospital, Prague, Czech Republic. Patients were recruited from the IBD Clinical and Research Centre, ISCARE IVF and First Faculty of Medicine of Charles University, Prague, Czech Republic. Controls: healthy blood donors Time-period: not specified	116	84	—	—	72	√	√	√	√	—	—	√	—	√	—
Malickova 2006 Czech Republic	Source and study period not reported. But, the study was conducted by Dept of Medicine, General Faculty Hospital and First Faculty of Medicine of Charles University, Prague, Czech Republic. Controls: healthy blood donors, source not specified Time-period: not specified	31	28	—	—	24	√	√	√	√	—	—	—	—	—	—
Rieder 2010 Germany	Cases: seen at the IBD center of the Department of Internal Medicine I, University of Regensburg, Regensburg, Germany. The sera belong to the serum repository of the German Competence Network IBD. (2000 – 2006) √ √ IC Excluded Controls: Healthy, UC and OGDs OGD: infectious colitis, pseudo-	363	130	—	74	257	√	√	√	√	√	√	√	√		

(*Continued*)

TABLE 1. (*Continued*)

	Source population	CD *n*	UC *n*	IBD *n*	OGD *n*	Healthy *n*	Serum Markers Measured						Clinical Outcomes Reported			
							ASCA	AMCA	ALCA	ACCA	Anti-L	Anti-C	Fistula/ Stricture	Perianal Disease	Disease Location	Surgery
	membranous colitis, diverticulitis, intestinal vasculitis, cirrhosis liver and chemotherapy induced colitis.															
Rieder 2011 Germany	Cases: Subgroup of the previous study. (IBD center of the Department of Internal Medicine I, University of Regensburg, Regensburg, Germany). (2000 – 2006). Patient charts reviewed in July 2007. A longitudinal cohort study. Contols: none	76	—	—	—	—	√	√	√	√	√	√	√	√	√	√
Rieder 2010 Germany	Cases: Subgroup of the previous study. (IBD center of the Department of Internal Medicine I, University of Regensburg, Regensburg, Germany). (2000 – 2006). Patient charts reviewed in July 2008. Contols: none	76	—	—	—	—	√	√	√	√	√	√	√	√	√	√
Seow 2009[a] Canada	Cases: Recruited from Mount Sinai Hospital and the Hospital for Sick Children, Toronto 2002-2006. Controls: Healthy controls.	517	301	818	—	97	√	√	√	√	√	√	√	√	√	√
Simondi 2008[a] Italy	Cases: IBD outpatients seen in gastroenterology clinic, 2006-2007 Healthy Controls: Blood donors from same hospital OGD: Celiac disease, IBS, colic diverticulosis, microscopic colitis, intestinal polyposis, GERD, chronic viral hepatitis, hepatic steatosis, chronic gastritis, peptic ulcer, or pancreatitis.	116	53	—	45	51	√	√	√	√	—	—	√	—	√	√
Papp 2008[a] Hungary	Cases: Recruited from four locations (5 centers); all were members of the Hungarian IBD Study Group (Budapest Semmelweis University 142 patients, Budapest Peterfi Hospital 76 patients, Debrecen University 117 patients, Szeged University 116 patients, and Veszprem Csolnoky Hospital 106 patients). Controls: healthy, age and gender matched, consecutive blood donors in Budapest and Debrecen. OGD controls: IBS, diverticulosis without inflammation. None of the controls had f/h/o IBD.	557	95	—	48	100	√	√	√	√	—	—	√	√	√	√

(*Continued*)

TABLE 1. (*Continued*)

	Source population	CD *n*	UC *n*	IBD *n*	OGD *n*	Healthy *n*	Serum Markers Measured						Clinical Outcomes Reported			
							ASCA	AMCA	ALCA	ACCA	Anti-L	Anti-C	Fistula/ Stricture	Perianal Disease	Disease Location	Surgery
Lakatos 2007[a] Hungary	Source and study period not reported. But, the study was conducted by Dept of Medicine, Semmelweis University, Budapest and Dept of Medicine, University of Debrecen, Debrecen. Controls: healthy blood donors, age and gender matched. Did not have any GI and/ or livers disease, and no f/h/o IBD.	376	—	—	—	100	√	√	√	√	—	—	√	—	√	—
Rejchrt 2008[a] Czech Republic	Cases: Source and study period not reported. But, the study was conducted by Dept of Medicine, University Teaching Hospital, Charles University in Prague. Time-period: not specified IC excluded. Controls: healthy blood donors.	89	31	—	—	50	—	—	√	√	—	—	√	—	√	—
Ferrante 2007[a] Belgium	Cases: were followed up at the IBD unit of the University Hospital in Leuven, Belgium. between 1998 and 2006. Controls: ethnically matched healthy control OGD controls: diverticulitis, Ischemic colitis, infectious colitis, and pseudo-membranous colitis.	913	272	1225	113	200	√	√	√	√	—	—	√	—	√	√
Henckaerts 2007[a] Belgium	Cases: were followed at the University Hospital Gasthuisberg, Leuven, a tertiary care referral centre. Time period: not specified. Controls: Healthy blood donors, OGDs. No f/h/o IBD or immune mediated disorders. OGD: Ischemic colitis, infectious colitis, and diverticulitis.	874	259	1163	113	199	√	√	√	√	—	—	√	√	√	—
Dotan 2006[a] Israel	Cases: Recruited at the Department of Gastroenterology and Liver Diseases, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel. Time-period: not specified Controls: Healthy blood donors OGD: celiac disease, IBS, diverticular disease, colonic polyps, pseudo-membranous colitis etc	124	106	—	61	40	√	—	√	√	—	—	√	—	√	√

Highlighted: Studies excluded from the meta-analysis because of patient population overlap; OGD, other gastrointestinal diseases; IBS, irritable bowel syndrome; f/h/o, family history of; IC, indeterminate colitis.
[a]Median, OGD.[b]Mean duration of disease not provided for OGD. Percent male and smoker not provided for OGD, or healthy controls.
[c]Dash, not reported.

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TABLE 2. Patient Characteristics of the Nine Studies Included in the Meta-analyses

Study Name	Mean Age at Study / Mean Age at Diagnosis (years)					Mean Duration of Disease[b] (years)			% Male[b]		% Ever Smoker[b]	
	CD	UC	IBD	OGD	Healthy	CD	UC	IBD	CD	UC	CD	UC
Koutroubakis et al, 2010	35[a]/–	46[a]/–	39[a]/–	62[a]/–	41[a]/–	5.3[a]	8.5[a]	7.1[a]	44	56	57	21
Malickova et al, 2010	28.9/–	39.7/–	—	—	26.1/–	6.3	—	—	41	58	—	—
Rieder F et al, 2010	35.7/ 28.3	39.3/ 32.3	—	60.7/–	43.9/–	5.6[a]	5.0[a]	—	47	61	—	—
Seow CH et al, 2009	33[a]/ 19[a]	39[a]/ 23[a]	—	—	45[a]/–	8.9[a]	8.9[a]	—	49	49	22	32
Simondi D et al, 2008	46/–	47/–	—	52.3/–	44.5/–	11.7	11.5	—	70	64	60	36
Papp M et al, 2008	36.4/ 28.3	39.7/ 30.8	—	—	36.6/–	8.1	8.9	—	47	46	40	20
Rejchrt S et al, 2008	—	—	—	—	—	—	—	—	50	42	—	—
Ferrante M et al, 2007	35[a]/ 22[a]	35[a]/ 27[a]	36[a]/24[a]	—	—	8.5	7.0	8.0	42	51	45	30
Dotan I et al, 2006	35.3/ 26.9	41.3/ 32.1	—	33.7	37.0	8.1	8.0	—	61	54	57	36

[a]Median, OGD.
[b]Mean duration of disease not provided for OGD. Percent male and smoker not provided for OGD, or healthy controls.Dash, not reported.

markers remain similar. Hence, the conclusions from the 14 studies are the same as that of the 9 studies included in the meta-analysis.

Patient Characteristics

The mean age of the IBD patients ranged from 29 to 47 years, with mean duration of disease ranging from 5 to 12 years (Table 2). One study included patients under 18 years of age,[27] but did not report the pediatric results separately from the adults. The healthy and other gastrointestinal disease controls were generally older than the CD and UC patients (Table 2).

Differentiation of Diagnosis

Overall, our analysis indicates that ASCA is the dominant factor in this anti-glycan marker panel in terms of the DOR for diagnostic differentiation, while no specific marker is prominent for disease behavior or surgery. ASCA has the highest sensitivity compared to the other anti-glycan markers for diagnosis of both CD (52.8%–56.6% vs. 15.0%–27.8%) and CD-related surgery (60.2% vs. 43.9%–47.3%) or complications (70.8% vs. 42.3%–54.5%). In terms of specificity, however, all single markers performed similarly (88%–95%; Table 3; Fig. 2). A combination of $\geq$2 anti-glycan markers performed better than individual markers for CD-related surgery, but was no better for complications or for differentiating CD from UC. Although the association of the number of positive anti-glycan markers with disease course could not be meta-analyzed, as stated above, it is important to note that an increasing number of positive anti-glycan antibodies was shown to be associated with penetrating phenotype, perianal disease, ileocolitis disease, and need for surgery.[27]

IBD vs. Healthy (Two Studies Included in Meta-analysis; Table 3)

Individually, ASCA had the highest sensitivity of 44% (specificity 96.4%), while ALCA had the highest specificity of 96.8% (sensitivity 15%). ASCA had the highest DOR for differentiating IBD from healthy (DOR 21.1; confidence interval [CI] 1.8–247.3).[9,27] Only one study[27] provided data for anti-L (DOR 13.4) and anti-C (DOR 3.6). No study reported the combination of markers for this outcome.

CD vs. Healthy (Six Studies Included in Meta-analysis; Table 3)

As shown in Table 3, individually ASCA had the highest sensitivity of 53.0% (specificity 70.4%), while ALCA had the highest specificity of 87.2% (sensitivity 26.0%). ASCA had the highest DOR for differentiating CD from healthy (DOR 2.7; CI 0.3–21.6).[6,26,28,29] Only one study[26] reported on anti-L (DOR 2.8) and anti-C (DOR 2.4). No study reported the combination markers. No study reported UC versus healthy.

CD vs. OGD (Other Gastrointestinal Disorders) (Four Studies Included in Meta-analysis; Table 3)

As shown in Table 3, for individual markers ASCA had the highest sensitivity of 52.8% (specificity 90.9%), while AMCA had the highest specificity of 94.7% but had the lowest sensitivity (17.4%). ASCA had the highest DOR for differentiating CD from OGD (DOR 10.3; CI 5.0–21.0).[6,26,28,29] Only one study[26] reported on anti-L (DOR 2.8) and anti-C (DOR 1.1). No study reported the combination markers. No study reported UC versus OGD.

CD vs. UC (Seven Studies Included in Meta-analysis; Table 3)

As shown in Table 3 and Figure 2, for individual markers ASCA had the highest sensitivity of 56.6%

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TABLE 3. Pooled Analyses of the Anti-glycan Antibody Markers for the Different Outcomes

Outcomes	Studies Included	Sensitivity (95% CI)	Specificity (95% CI)	Diagnostic Odds Ratio DOR (Lower, Upper)	I^2 %	HG (*P*-value)
ASCA	6,9,17,26,27,28,29	56.6 (51.9, 61.3)	88.1 (85.8, 90.0)	10.2 (7.7, 13.7)	49.3	0.130
AMCA	6,9,26,27,28	18.1 (11.7, 26.9)	92.3 (84.8, 96.2)	2.6 (1.7, 4.2)	68.2	0.051
ALCA	6,9,26,27,28,29,31	23.7 (17.7, 30.9)	91.9 (87.9, 94.7)	3.5 (2.7, 4.5)	0.0	0.838
ACCA	6,9,26,27,28	15.7 (10.7, 22.4)	92.3 (85.3, 96.1)	2.1 (1.5, 2.9)	42.7	0.264
Anti-L	26,27	21.5 (15.0, 29.9)	95.1 (89.6, 97.8)	5.3 (3.3, 8.6)	0.0	0.444
Anti-C	26,27	16.4 (6.4, 35.9)	94.9 (79.5, 98.9)	3.5 (2.1, 5.7)	0.0	0.308
CD vs. OGD						
ASCA	6,26,28,29	52.8 (44.4, 61.1)	90.9 (77.2, 96.7)	10.3 (5.0, 21.0)	59	0.181
AMCA	26,28,29	17.4 (9.2, 30.5)	94.7 (86.6, 98.0)	4.7 (2.2, 10.2)	0.0	0.746
ALCA	6,26,28,29	27.8 (15.9, 43.8)	91.7 (81.8, 96.5)	4.8 (2.7, 8.4)	0.0	0.945
ACCA	6,26,28,29	21.6 (12.0, 35.6)	90.9 (78.3, 96.5)	3.4 (0.8, 13.3)	86.5	0.002
CD vs. healthy						
ASCA	6,26,28,29	53.0 (44.6, 61.3)	70.4 (27.6, 93.7)	2.7 (0.3, 21.6)	98	0.000
AMCA	6,26,28	17.4 (9.2, 30.5)	72.4 (2.1, 99.7)	0.6 (0.1, 93.3)	98.1	0.000
ALCA	6,26,28,29,30,31	26.0 (17.5, 36.8)	87.2 (56.2, 97.3)	2.3 (0.8, 6.9)	92.3	0.000
ACCA	6,26,28,31	15.0 (9.4, 22.9)	81.0 (22.2, 98.5)	0.7 (0.1, 7.2)	96.9	0.000
IBD vs. healthy						
ASCA	9,27	44.0 (41.8, 46.2)	96.4 (71.5, 99.7)	21.1 (1.8, 247.3)	0.0	0.001
AMCA	9,27	15.4 (4.8, 39.8)	94.3 (86.5, 97.7)	3.8 (2.4, 6.2)	0.0	0.634
ALCA	9,27	15.0 (13.5, 16.6)	96.8 (87.7, 99.2)	5.3 (1.3,21.8)	0.0	0.046
ACCA	9,27	11.4 (3.7, 30.4)	92.5 (70.9, 98.4)	1.5 (1.0, 2.2)	0.0	0.504
Had complication						
ASCA	9,17,26	70.8 (67.6, 73.9)	48.5 (40.5, 56.6)	2.4 (1.9, 3.1)	0.0	0.711
AMCA	9,26	54.5 (14.7, 89.3)	66.1 (22.7, 92.8)	2.4 (1.8, 3.2)	0.0	0.894
ALCA	9,17,26	42.3 (15.0, 75.3)	65.3 (34.5, 87.0)	1.5 (1.1, 1.9)	0.0	0.810
ACCA	9,17,26	43.3 (9.0, 85.6)	75.1 (35.2, 94.4)	2.7 (2.0, 3.6)	20.4	0.533
Had surgery						
ASCA	6,9,27	60.2 (48.6, 70.7)	57.3 (47.6, 66.4)	2.0 (1.6, 2.4)	0.0	0.708
AMCA	6,9,27	47.3 (26.6, 68.9)	65.4 (48.5, 79.2)	1.7 (1.0, 2.9)	90.6	0.005
ALCA	6,9,27	43.9 (22.6, 67.6)	60.6 (45.5, 73.9)	1.3 (92.0, 73.2)	76.7	0.117
ACCA	6,9,27	46.1 (19.2, 75.4)	67.3 (48.6, 81.7)	2.0 (1.6, 2.4)	26.8	0.505
Combination ($\geq$2 markers)						
CD vs. UC	17,26,28	41.5 (26.8, 57.9)	92.8 (84.4, 96.8)	10.2 (5.6, 18.5)	62.1	0.267
Needed surgery	9,27	61.5 (51.6,70.6)	63.8 (54.6, 72.0)	2.8 (2.2, 3.6)	0.0	0.917
Had complication	9,27	62.1 (48.4, 74.1)	61.8 (41.8, 78.6)	2.8 (2.2, 3.7)	0.0	0.339

HG, heterogeneity, OGD, other gastrointestinal diseases.

(specificity 88.1%) while Anti-L had the highest specificity of 95.1% (sensitivity 21.5%). ASCA had the highest DOR for differentiating CD from UC (DOR 10.2; 95% CI 7.7–13.7; seven studies[6,9,17,26–29]; Fig. 2). Anti-L had the second highest DOR for differentiating CD from UC (DOR 5.3; CI 3.3–8.6; two studies).[26,27] The DORs for the other markers were also significantly greater than one: Anti-C, 3.5 (CI 2.1–5.7); ALCA, 3.5 (CI 2.7–4.5); AMCA, 2.6 (CI 1.7–4.2); and ACCA, 2.1 (CI 1.5–2.9). When a combination of positivity for $\geq$2 markers versus $\leq$1 was used to distinguish CD from UC, the DOR was 10.2 (CI 5.6–18.5; sensitivity 41.5%; specificity 92.8%; three studies).[17,26,28]

A number of studies have reported marginal to no improvement in differentiation of CD from UC by adding other anti-glycan markers to gASCA and pANCA,[9,30] while others[26] reported that the addition of Anti-L and Anti-C to gASCA/pANCA significantly increased the discriminatory capacity for CD versus UC. The combination of two or

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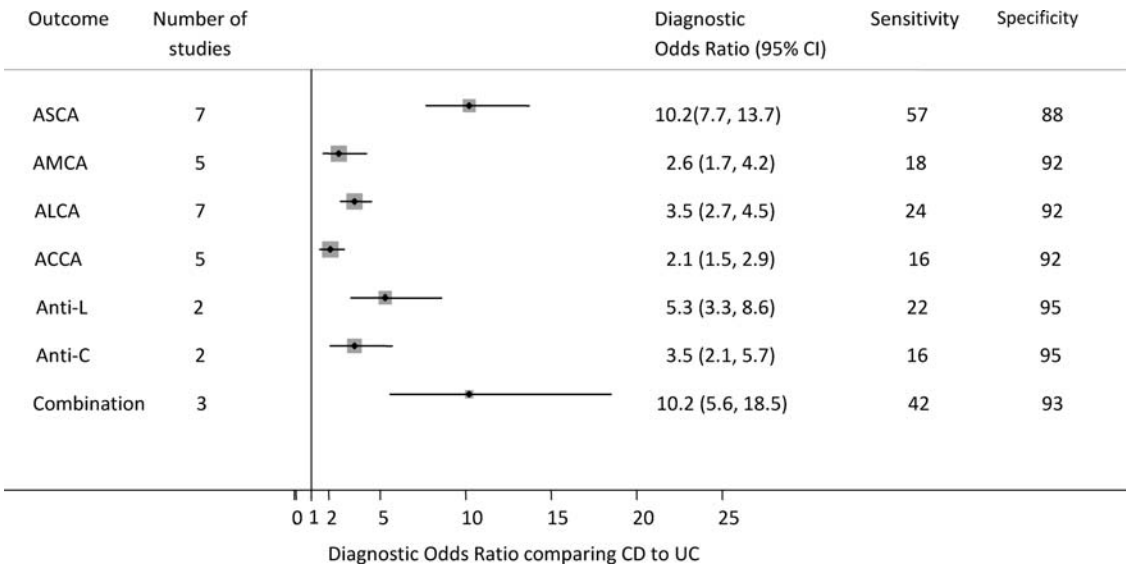

FIGURE 2. Forest plot of pooled anti-glycan biomarkers for differentiating CD from UC.

more of these markers was better than any of the markers alone, although we could not tell which markers specifically contributed to the combination. On the other hand, it may not be necessary to specify the particular marker in the combination because of the low sensitivity of ALCA, ACCA, and AMCA.

Disease Phenotype

Of the 14 studies included in our systematic review, disease phenotype, (disease behavior and location) was defined by the Montreal Classification in six studies,[22,24,25,27,28,30] Vienna classification in two studies,[17,29] both Vienna and Montreal in four studies,[6,9,21,26] and was not specified in two studies.[23,31]

Disease Behavior

All nine studies included in the meta-analysis reported disease behavior, but only three studies reported their results in the quantitative detail necessary for inclusion in a meta-analysis.[9,17,26] All other studies reported the data qualitatively or gave only the direction of the relationship with a *P*-value. For the meta-analyses, we combined stricturing and penetrating/fistulizing disease into the category of complication.[9] One study was excluded from the meta-analysis of combination of markers because it included OmpC (non-anti-glycan) in the combination.[6] As shown in Table 3 and Figure 3, for individual markers ASCA had the highest sensitivity of 70.8% (specificity 48.5%), while ACCA had the highest specificity of 75.1% (sensitivity 43.3%). ACCA had the highest DOR of 2.7 (CI

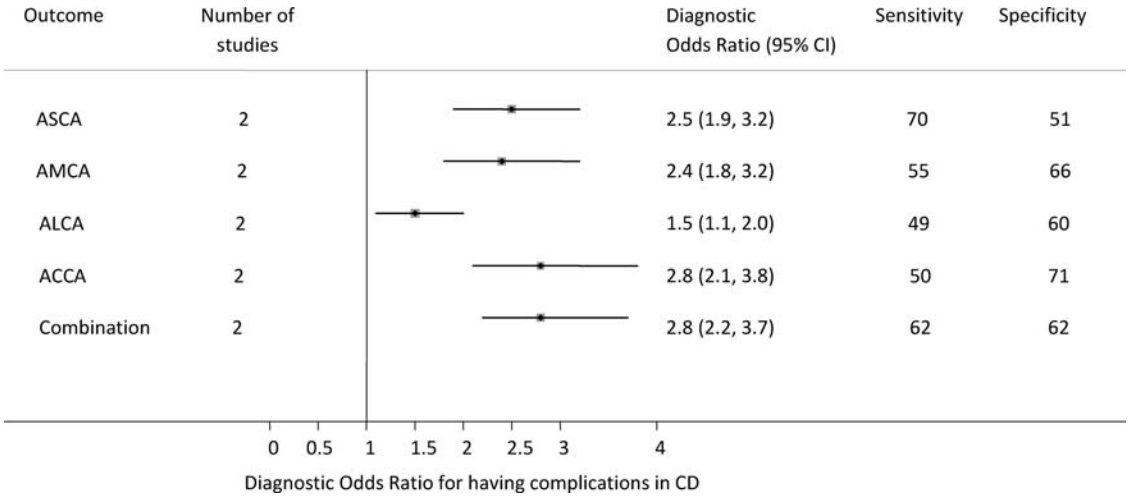

FIGURE 3. Forest plot of pooled anti-glycan markers for having complications.

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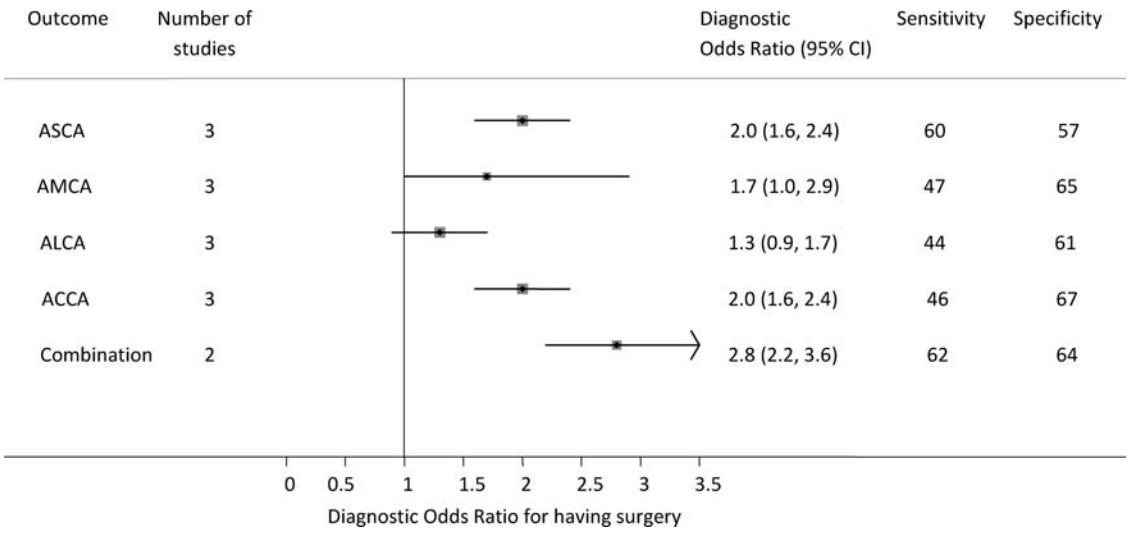

FIGURE 4. Forest plot of pooled anti-glycan markers for having surgery.

2.0–3.6). None of the included studies provided data for this outcome for anti-L and anti-C. The pooled DOR for complications when a combination of $\geq$2 markers was used was 2.8 (CI 2.2–3.7; 2 studies),[9,27] higher than any single marker alone (2.8 vs. 2.0), which shows a numerical, although not statistical tendency toward a higher chance of having a CD-related surgery.

In addition to the positivity(?), the levels of anti-glycan markers have also been analyzed for their association with disease behaviors or need for surgery. Higher serum levels of gASCA have been associated with stricturing and/or penetrating behavior in literature.[27,29,30,32] The relationship with rest of the anti-glycan markers is less clear, ranging from no association[32] to differing association.[17,27,29] A recently published review by Lakatos et al[33] reported that the likelihood of complicated CD behavior and CD-related surgery increases with the quartile score of the markers.

The other studies that documented the outcome, but could not be included in the meta-analysis, reported that the patients with stricturing or penetrating disease were more likely to have more than one positive anti-glycan marker.[6,9,17,26,27] Considering individual markers, there was an inconsistent association between CD behavior and the different anti-glycan markers in the studies. Rejchrt et al[31] found that the ALCA and ACCA positivity did not differ with disease phenotype or location. Rieder et al[24] reported a higher positivity for ASCA, AMCA, and Anti-L antibody markers in naïve patients (defined as patients with no complications [fistula, stenosis] or surgery before or within 20 days of sample procurement) progressing to a first complication event or IBD-related surgery. The median time to complication as well as surgery was 11.6 months. They also reported a higher likelihood for early progression to a disease event in patients positive for ASCA, AMCA, ACCA, and Anti-L. Koutroubakis et al[28] found ASCA and ALCA to be significantly associated with disease phenotype, but no association with AMCA and ACCA. Rieder et al[24] reported that CD patients positive for at least two out of the six anti-glycan markers had a higher likelihood for complications and a more severe disease course. Our review of the literature suggests similarly that an increasing number of positive markers was associated with more aggressive disease and CD-related surgery.[6,9,17,26,27] Longer disease duration (see more details below), ileal involvement (see more in Disease Location, below), and the number of positive serological markers have been reported as independent predictors of stricturing/penetrating disease behavior.[6]

Age at Diagnosis and Disease Duration

Seow et al[27] reported an increasing number of positive antibodies to be associated with early age of CD diagnosis ($P = 0.0004$) and longer disease duration ($P = 0.005$). They also found an independent association between gASCA and early disease onset (OR 1.74, 95% CI 1.12–2.52; $P = 0.0035$) and longer disease duration (OR 2.63, 95% CI 1.09–6.34; $P = 0.03$). Ferrante et al[9] reported significantly longer disease duration in patients who were positive for gASCA, ACCA, AMCA, or OmpC (but not ALCA) compared to those who were negative for these serological markers. However, gASCA ($P < 0.0001$) and ALCA ($P = 0.012$) were shown by Papp et al[6] to be associated with younger age at onset, but not with disease duration (the percentage of serological marker positivity not different between patients with $<$10 and $\geq$10 years disease duration). Malickova et al[30] reported a different frequency of gASCA between the four different groups of CD

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patients in their study, divided according to disease duration, but not variation of AMCA, ALCA, and ACCA with disease duration.

Surgery

Of seven studies reported,[6,9,17,26–29] three qualified for the meta-analysis.[6,9,27] As shown in Table 3 and Figure 4, individually ASCA had the highest sensitivity of 60.2% (specificity 57.3%) for surgery, with ACCA having the highest specificity of 67.3% (sensitivity 46.1%). The DOR for CD-related surgery was similar for ASCA and ACCA, being 2.0 (CI 1.6–2.4).[6,9,27] When positivity for $\geq$2 markers was used for the outcome, the DOR was 2.8 (CI 2.2–3.6),[9,27] which was higher than any of the individual markers.

Disease Location

All nine studies included in the meta-analysis reported this outcome, but we could not do a meta-analysis for this outcome as the data were not in a retrievable form for meta-analysis. Independently, in the included studies it was found that the relationship between positivity of anti-glycan markers and disease location was highly inconsistent. Apart from gASCA, which was found to be consistently associated with ileal[26,28,29] or ileocolonic CD,[27,30] the association of other anti-glycan markers with disease localization in CD varied, to almost no association between ACCA, ALCA, or AMCA and CD localization.[30] Seow et al[27] reported that only gASCA IgA (20.4 vs. 6.0%, $P < 0.001$) and anti-L (14.3 vs. 3.3%, $P < 0.001$) were able to differentiate isolated inflammatory colonic CD from UC. However, Ferrante et al[9] demonstrated gASCA, ALCA, ACCA, and OmpC to be independently associated with ileal involvement (P-values of 0.010, 0.033, 0.044, and 0.044, respectively). Perianal disease is often seen as a different subset of CD than abdominal disease. The presence of perianal disease alone (B1p) was reported to be associated with a higher frequency of glycan antibodies compared with the absence of complicated disease behavior (B1).[26] AMCA and anti-C were reported to be independently associated with perianal disease.[27]

ASCA-negative Patients

Another benefit of adding these novel anti-glycan antibody markers to gASCA alone may be to diagnose CD in patients otherwise negative for gASCA. Studies have reported 32%–56% of their gASCA-negative patients positive for at least one of the three anti-glycan antibody markers AMCA, ALCA, and ACCA.[9,17,29,30] The information could not be meta-analyzed due to lack of available data from the included studies.

Association of Genotypes with Serological Anti-glycan Markers

One of the most intriguing observations regarding anti-glycan IBD biomarkers is a potential association of these serological markers with the genetic markers, the variants of IBD susceptible genes. At least four studies,[6,21,22,34] first reported by Henckaerts et al[21] and later by Papp et al[6] and Lakatos et al[22,34] examined the influence of mutations in several IBD susceptible genes on the development of anti-glycan in IBD, including NOD2/CARD15, NOD1/CARD4, CARDINAL/CARD8, Toll-like receptors (TLRs; TLR1, TLR2, TLR4, and TLR6), DLG5, and DEFB1.

In Henckaerts et al's study,[21] gASCA or ALCA positivity in CD patients with at least one NOD2/CARD15 variant was significantly more frequent than those with no mutation (gASCA: 66.1% vs. 51.5%, $P < 0.0001$; ALCA: 43.3% vs. 34.9%, $P = 0.018$). The gASCA titers were also higher in CD patients with NOD2/CARD15 mutations than those with no mutation (85.7 vs. 51.8 enzyme-linked immunosorbent assay [ELISA] units, $P < 0.0001$). A remarkably similar ASCA association with NOD2/CARD15 was reported by Papp et al.[6] In addition to ASCA, the positivity of AMCA in CD patient with NOD2/CARD15 mutations was 2-fold higher than those with no wildtype (WT) alleles (18.8% vs. 9.7%; $P = 0.009$).[6] More intriguingly, both studies[6,21] observed a gene dosage effect when positivities of anti-glycan antibodies in CD patients carrying 0, 1, and 2 NOD2/CARD15 variants were compared: Positivity frequencies of anti-glycan markers (gASCA, ALCA, AMCA) increased gradually with increasing number of NOD2/CARD15 mutations (see review[35]).

CD patients with a NOD1/CARD4 GG-indel allele exhibited significantly higher gASCA prevalence when compared with those with the WT allele (63.8% vs. 55.2%, $P = 0.014$).[21] In contrast to NOD2, an inverse gene dosage effect of TLR4 on ACCA was observed by Henckaerts et al[21]: The prevalence of ACCA in CD patients with 0, 1, and 2 TLR4 variants is 34.9%, 24.8%, and 9.1%, respectively. Two DEDB1 variants, G20A and C44G, were also found to be inversely associated with the positivity of anti-glycan antibodies.[22] It is necessary to note that there is inconsistency among different studies with regard to the association of genetic markers with anti-glycan markers. For example, in studies by Lakatos et al,[22,34] no significant association was found of NOD2, TLR4, NOD1, and DLG5 with the positivity of any anti-glycan markers.

Stability of Markers over Time

We found three published studies[24–26] by the same group on this subject, one of which was recently published. They reported substantial changes in the levels of the

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antibodies in their patients over time, but the status of the markers remained stable over time in terms of positivity or negativity for an anti-glycan biomarker antibody. The authors attributed the fluctuations to unidentified clinical factors, genotypes, or natural changes over time. They also sent out a word of caution on using the Quartile Serum Score for disease stratification due to these strong fluctuations in marker levels.

Applicability of Anti-glycan Markers in UC

Anti-glycan antibodies were generally considered markers specific for CD and thus useful for differentiating CD from UC. Malickova et al[30] reported that none of the assessed anti-glycan markers was predictive of colonic CD or UC and did not report more detailed data for this reason. Papp et al[6] also reported that no clinically important serotype-phenotype associations were seen in UC, for which the data were not presented. Ferrante et al[9] reported that gASCA, ACCA, and AMCA were inversely associated with UC-like disease behavior among CD patients, but no data were reported.

This is the first study to employ meta-analytical techniques to assess the diagnostic and predictability value of these anti-glycan markers in IBD. Although narrative reviews have been previously published,[18,32–35] much of the existing literature on the subject is based on individual studies. Inevitable biases are introduced by pooling different observational studies, reflected by the statistical heterogeneity present throughout the analysis. All the included studies in this systematic review and meta-analysis were retrospective in nature. Although two studies[24,25] claimed to have prospectively analyzed the patients, they did retrospective chart reviews to update their data points over time, and their results may be considered different from the other included cross-sectional studies. The observed heterogeneity in our analysis could be due to various sources including different cutoffs used for marker positivity, disease duration, various therapies, age at diagnosis, smoking habit, role of family history, sex, and body mass index (BMI). The source of control populations in the different studies was not clearly defined, and it was not explicitly stated whether the healthy controls and patients had been screened for IBD before or during recruitment. Most of studies included in our review presumably studied Caucasian populations (European subcontinent and Canada), except one study from Israel,[17] which studied a Jewish population, and the yield for the different markers could vary according to ethnicity.

RESEARCH LIMITATION/FUTURE STUDIES NEEDED

We found a number of research limitations during our review of the existing literature on the role of anti-glycan markers in IBD, which could potentially pave the path for the future studies on this subject.

Need for Prospective Studies

The retrospective design of the studies precludes any analysis of these markers for their predictive ability, for diagnostic outcomes as well as disease course. The statistical analysis in these retrospective studies is influenced by the composition of the study population, which was of patients already diagnosed, and therefore the performance of these markers is influenced by the pretest probability.

Influence of IBD Genetic Markers / IBD Susceptible Genes on Serological Anti-glycan Biomarkers

As described above, although data are limited, there is clear indication that genetic markers have significant influence on the prevalence and/or levels of anti-glycan markers in CD patients. Current inconsistency between different studies may arise from the differences in samples sizes and/or ethnic backgrounds of study cohorts. In the era when a fast-expanding number of IBD susceptible genes (both in CD and UC) have been identified by genome-wide association studies (GWAS),[36–38] future studies are absolutely necessary to analyze the association of these genetic markers with serological biomarkers (both anti-glycan and other biomarkers) in both CD and UC. This can be achieved only with much larger patient cohorts than what have been done currently, through close collaboration between major IBD centers, or the NIDDK IBD Genetic Consortium, a highly successful study group responsible for identifying most of the major IBD susceptible genes[36–38] (see http://medicine.yale.edu/intmed/ibdgc/index.aspx). A combination of genetic and serological biomarkers, if successful, may potentially revolutionize the way IBD diagnosis and management are currently performed.

Benefit of Marker Combination

The included studies combined various markers for knowing the combined efficacy, but did not specifically name the combinations, and as such we would not know which combination works the best or if there is some major driving marker among all the measured markers. ASCA had greatest predictive power. Since the incremental benefit of multiple markers was small compared to ASCA alone, it is important to know if ASCA was included in the combination of markers and if addition of other markers provided only marginal improvement in predictability compared to ASCA alone.

Influence of Ethnic Backgrounds

We have evidence that showed significant sensitivity and specificity of these anti-glycan markers in African-Americans compared with those of Caucasians (DDW

Abstract #1041828). However, there is no published study aimed at comparing the diagnostic values of these markers or their association with disease complication among subgroups of subjects with different race/ethnicity/ancestry.

Pediatric Population

No data are provided on the performance of this marker group in the pediatric age group. Only one study[27] that met the inclusion criteria included a pediatric population, but did not report them separately from adults, which could also be a source of potential heterogeneity in the study.

Marker Stability

Only three studies, from the same group,[24–26] looked into the stability of these markers over time, and more studies would be needed to truly prospectively follow these markers over time.

Effect of Therapy on Marker Positivity and Levels

We do not know whether these markers change, in positivity or level, with intervention, medical or surgical, which needs to be addressed in prospective studies.

Anti-glycan markers in Indeterminate Colitis (IC)

IC, comprising 10%–15% of all IBD patients,[39,40] has either not been reported at all or purposefully excluded, citing low patient numbers.

In conclusion, ASCA had the highest diagnostic value among individual anti-glycan markers, while ACCA had the highest association with complications. For risk of surgery, ASCA and ACCA perform equally well. Although a combination of $\geq$2 markers was reported to be better in diagnosis and prognosis in most of the individual studies, we found the combination performing slightly better than any individual marker in our meta-analysis.

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