



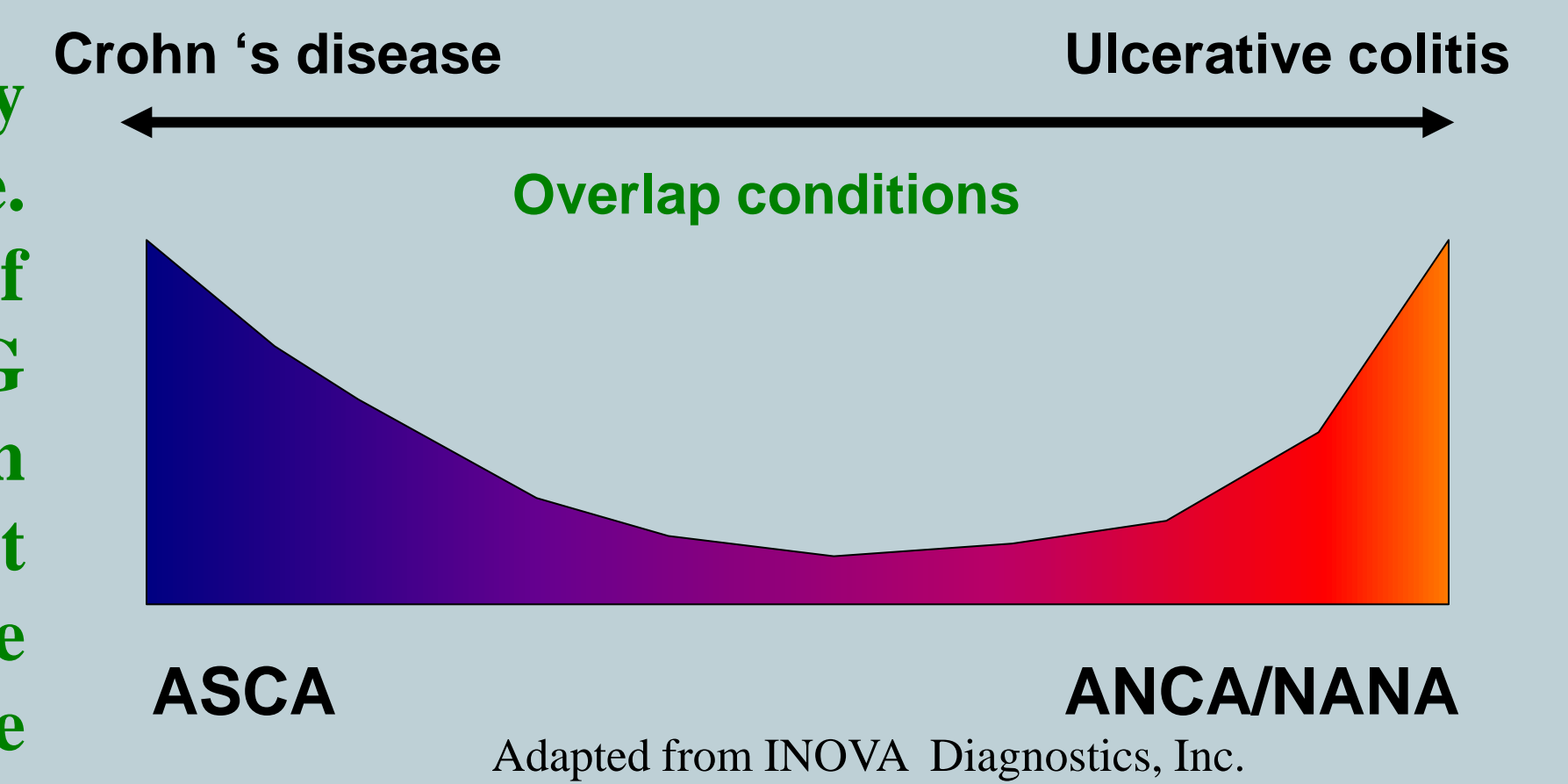
EVALUATION OF A NEW SPECIFIC MARKER FOR THE DIAGNOSIS OF PATIENTS WITH CROHN'S DISEASE: THE ANTI-OMP ANTIBODIES

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BACKGROUND

Crohn's disease (CD) and ulcerative colitis (UC) are the two major forms of inflammatory bowel disease (IBD). Since many symptoms of Crohn's disease and ulcerative colitis are similar, diagnosis is often difficult, time consuming and invasive. Approximately 10-12% of cases are not initially classifiable and are referred to as "indeterminate colitis". Over time, about half of these patients are eventually classified as CD or UC. Anti-*Scharomyces cerevisiae* antibodies (ASCA) of both classes IgG and IgA have been found to be significantly more prevalent in patients with Crohn's disease compared to patients with ulcerative colitis or healthy controls. Some Crohn's disease patients have been found to have also antibodies directed against antigens derived from bacteria as those targeting the outer membrane protein C (OMP C) of *E.coli*. Antibodies to these antigens appear to be present in a subgroup of ASCA-negative Crohn's disease patients and correlates with a more complicated disease phenotype.



MATERIALS

A retrospective study was conducted using 125 frozen sera.

Group		
Crohn's Disease	24	
Ulcerative Colitis	22	
Controls:	79	
coeliac disease	22	
HCV	10	
autoimmune hepatitis	10	
Hyper IgG (>20g/l)	9	
Hyper IgA (>10g/l)	10	
RF (>500 U)	11	
Healthy subjects	7	

METHODS

ELISA technique. Anti-OMPc antibodies were measured using the QUANTA Lite™ OMP Plus ELISA (Inova diagnostics, Inc. San Diego, CA; commercialized in Switzerland by Ruwag AG). INOVA assay uses a proprietary mixture of Outer Membrane and associated proteins from 2 different anaerobic bacteria isolated from 2 different patients with Crohn's disease.

Pre-diluted controls and diluted (1:101) patient sera were added in duplicate to separate well and incubated for 30 min. at room temperature (RT), allowing any anti-OMP antibodies present to bind to the purified OMP Plus antigen bound to the surface of the polystyrene microwell ELISA plate. Unbound sample was washed away and enzyme (HRP) labelled anti-human IgA antibody (goat) was added to each well and incubated for 30 min. at RT. After washing, the remaining enzyme activity was measured by adding the TMB chromogenic substrate for 30 min. The optical density (OD) at 450 nm was measured using a microplate reader. The average OD for each set of duplicates was first determined.

The reactivity for each sample could then be calculated by dividing the average OD of the sample by the average OD of the OMP Plus ELISA low positive control. The result was multiplied by the number of units assigned to the OMP Plus ELISA low positive control found on the label which is 25 units.

The sera were classified as:

- negative (< 20 Units),
- equivocal (20.1 -24.9 Units)
- positive (>25 Units).

All sera were stocked at -80 °C and thawed just before analyses were performed.

RESULTS :

- In the 24 Crohn's disease sera tested, 7 (29 %) were anti-OMPc IgA positive (Tab.1). In 101 Crohn's negative patients 11 (10.9 %) were anti-OMPc IgA positive (Fig.1 and Tab. 1). Among them, 4 were related to coeliac disease. Taking equivocal results as positive results, anti-OMPc sensitivity was still rather low at 33% but specificity reached 85% (Fig.2)

Tab 1 Anti-OMPc IgA results

Group	POS (%)	equivocal	Neg
Crohn's disease	7 (29)	1	16
Ulcerative colitis	1 (4.5)	2	19
Coeliac disease	4 (18)	1	17
Autoimmune hepatitis	1 (10)	0	9
HCV	1 (10)	0	9
IgG > 20g/l	1 (11)	0	9
IgA > 10g/l	2 (20)	1	7
RF > 500	1 (9)	0	10
Healthy subjects	0 (0)	0	7

Fig.1 Quantitative anti-OMPc IgA results

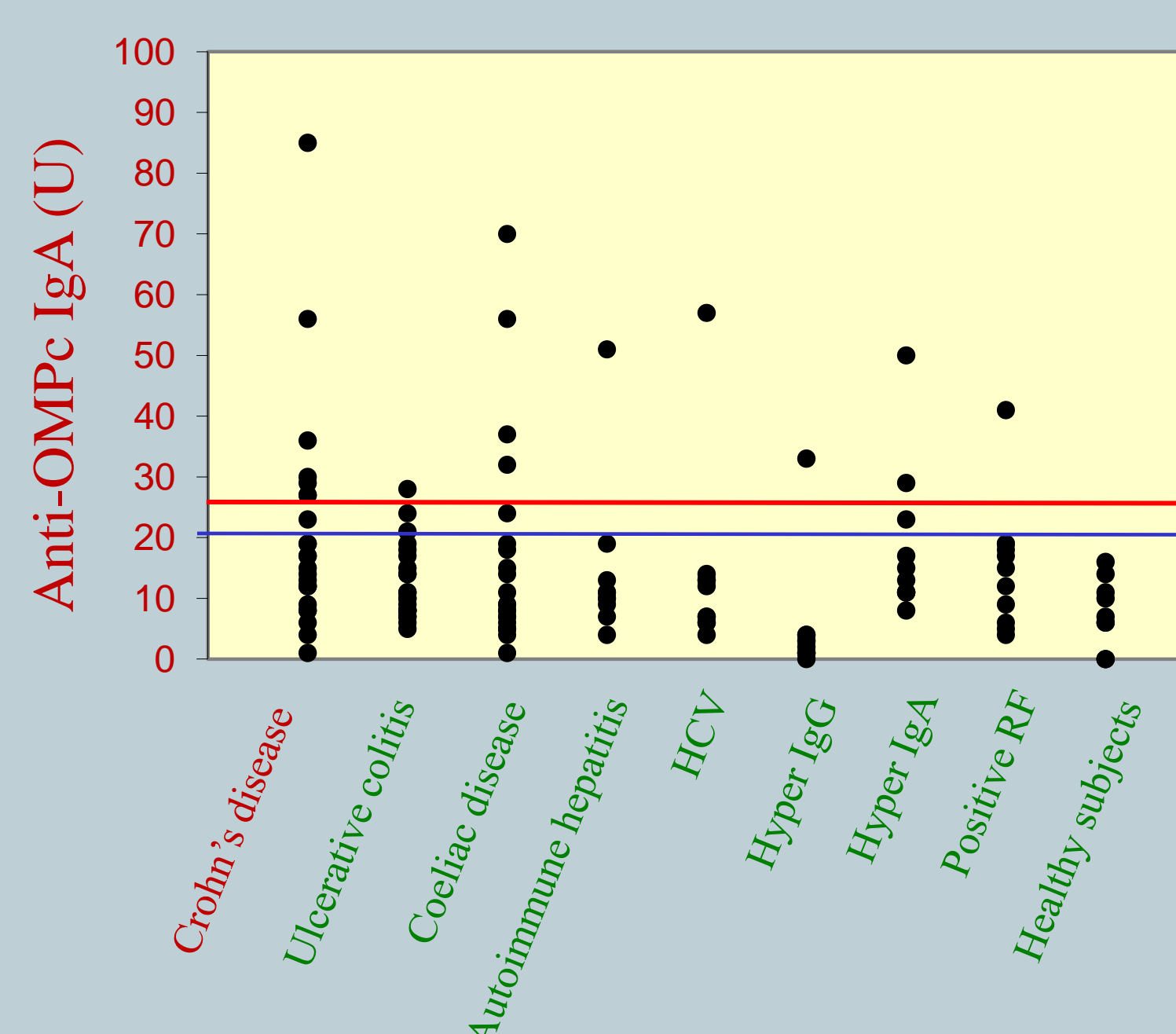


Fig.2 Anti-OMPc IgA sensitivity and specificity

Crohn	OMPc IgA		
	pos	neg	
pos	8	16	24
neg	15	86	101
	23	102	125

Sens. 33 %
Spéc. 85 %
VPP : 35 %
VPN : 84 %

- As ASCA is actually used in the serodiagnosis of CD, we compared anti-OMPc results with ASCA (IgG or IgA) results in 3 different IBD. We demonstrated a slight increase (4%) of sensitivity by using both markers with one of them being positive (Tab. 2). Concordances were much better between negative than positive results (Fig 3). On the other hand, when we looked for ASCA IgG and IgA and anti-OMPc IgA positive results, we observed a very interesting specificity value reaching 95% but a sensitivity falling down at 17% (Fig 4).

Tab 2 Reactions with other inflammatory bowel diseases

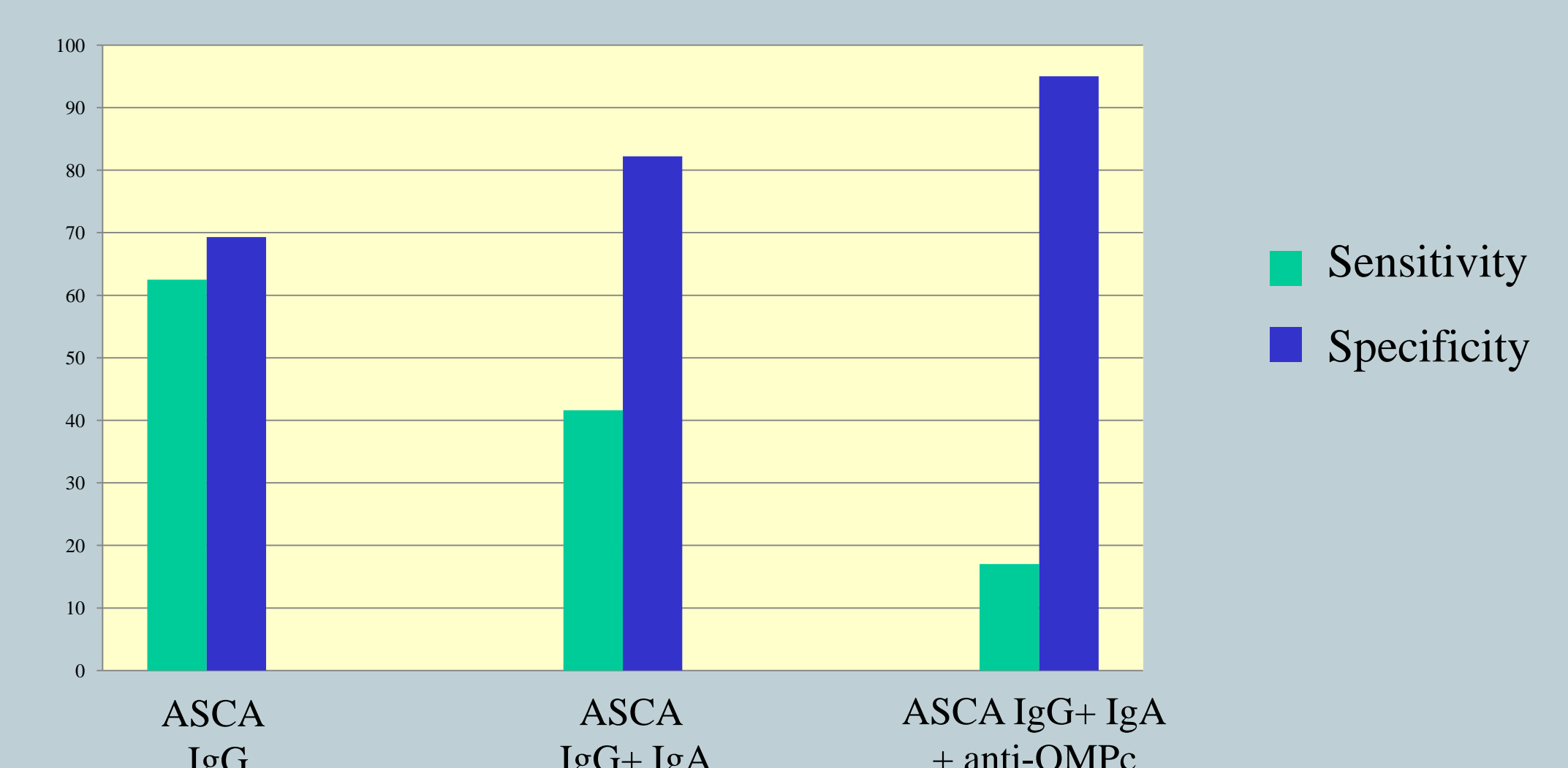
		Crohn's disease	Ulcerative colitis	Coeliac disease
ASCA (IgG or IgA)	Sensitivity	58 %	18 %	63 %
	Specificity	74 %	65 %	71 %
OMPc IgA	Sensitivity	33 %	14 %	22 %
	Specificity	85 %	83 %	86 %
ASCA or OMPc	Sensitivity	62.5 %	31 %	63 %
	Specificity	63 %	63 %	67 %

Fig 3 Comparison ASCA versus anti-OMPc

Anti-OMPc	ASCA (IgG or IgA)		
	pos	neg	
pos	19	4	23
neg	29	73	102
	48	77	125

positive concordances : 39%
negative concordances : 95%
Total concordances : 74%

Fig 4 Incremental value of specificity by adding respectively ASCA IgG plus IgA plus OMPc in testing for Crohn's disease



- In the 24 CD patients tested, 9 were seronegative (ASCA and anti-OMPc) and 1 (4%) was anti-OMPc IgA positive alone

CONCLUSIONS :

- The anti-OMPc IgA antibody assay is a new additional tool for the diagnosis of Crohn's disease. Our results showed that OMPc IgA antibodies were more prevalent in CD, although there was some overlap with coeliac disease
- As its sensitivity seems not to be very high, this test should not be used alone as a screening test. To improve the sensitivity for CD screening, anti-OMPc IgA assay should be used in association with ASCA detection
- Testing with ASCA IgG and IgA and OMPc antibodies improves specificity (when all 3 results are negative) for Crohn's disease up to 95%. Thus results including negative ASCA and anti-OMPc may be used to exclude a Crohn's disease
- Inclusion of anti-OMPc IgA assay offers additive diagnostic informations for Crohn's disease over ASCA, but further studies are needed until laboratory tests will replace the biopsy proven diagnosis of IBD