

Evaluation of three *Campylobacter pylori* antigen preparations for screening sera from patients undergoing endoscopy

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SUMMARY A surface antigen (SA), acid glycine extract (AGE), and urease preparation (UP) were evaluated using sera from patients undergoing endoscopy and from subjects with gastric or duodenal ulcers. Sera were tested for the presence of IgG and IgA antibodies by a conventional indirect enzyme linked immunosorbent assay (ELISA). In patients with confirmed *Campylobacter pylori* associated gastritis, raised IgG antibody titres were indicated by absorbance values of ≥ 500 , ≥ 500 , and ≥ 1500 for the SA, AGE, and UP, respectively. Corresponding values for the IgA assay were ≥ 500 , ≥ 500 , and ≥ 1000 . The specificity of the IgG assays were 94%, 92%, and 90% for the AGE, SA, and UP, respectively. In contrast, the UP was the most sensitive (97%); the other two antigen preparations gave values of 82%. In the IgA assay the UP showed the greatest specificity (90%) and sensitivity (90%). The predictive value for a true positive for the IgG assay was the same for all antigens (93%), whereas the UP gave a predictive value for a true negative of 96% compared with 79% for the other two antigen preparations. Of the patients with gastric or duodenal ulcers, raised antibody titres to SA were found in 72% (IgG) and 73% (IgA), to AGE in 75% (IgG) and 63% (IgA), and to UP in 77% (IgG) and 75% (IgA). The use of a urease antigen preparation to determine IgG antibody is recommended for screening patients undergoing endoscopy.

Of the methods used to study the antibody response to *Campylobacter pylori*, one of the most common is enzyme linked immunosorbent assay (ELISA). Several antigen preparations have been used with this method. In a previous study we used a surface antigen,¹ other workers have described an acid-glycine extract,^{2,3} and more recently an antigen preparation rich in *C pylori* urease has been used.⁴ The present study was undertaken to evaluate these three antigen preparations and to determine their efficacy in the screening of patients undergoing endoscopy.

Material and methods

Three *C pylori* antigen preparations were made as described previously: surface antigen (SA)¹ used at 0.5 mg/l, acid glycine extract (AGE)² used at 2.5 mg/l, and a urease preparation (UP)⁴ used at 10 mg/l.

The sera tested comprised 115 serum samples from patients undergoing endoscopy and 84 serum samples from patients with gastric or duodenal ulcers.

Serum samples were diluted 1/100 for the IgA assays

and 1/1000 for the IgG assay. These were tested by a conventional indirect ELISA using a peroxidase-H₂O₂-ortho phenylenediamine detection system and read at a wavelength of 490 nm.⁵ All results were recorded and expressed as absorbance $\times 1000$. Positive and negative control sera were included in each batch of tests.

Results

IgG antibody responses in both sets of patients are shown in table 1. Absorbance values of ≥ 500 for SA, ≥ 500 for AGE, and ≥ 1500 for UP were selected as indicative of a raised IgG titre. The percentage of patients with gastritis, *C pylori*, and raised antibody titres was 82% for SA and AGE and 97% for UP. In contrast, the percentage of patients without gastritis, no *C pylori*, but with raised titres was 8% for SA, 6% for AGE, and 10% for UP. The proportion of raised titres in those patients with gastric or duodenal ulcers was 72% for SA, 75% for AGE, and 77% for UP.

IgA antibody responses in both sets of patients are shown in table 2. Absorbance values indicative of raised IgA titres were ≥ 500 for SA and AGE and

Table 1 Distribution of IgG antibody responses determined using three *C. pylori* antigen preparations

Clinical group	Antigen preparations	No of samples giving absorbance values (× 1000)				
		< 500	< 750	< 1000	< 1500	≥ 1500
Gastritis* and <i>C. pylori</i> detected† (n = 67)	SA	12	13	11	19	12
	AGE	12	12	13	13	17
	UP	0	0	2	0	65
No gastritis and <i>C. pylori</i> not detected (n = 48)	SA	44	2	0	2	0
	AGE	45	2	1	0	0
	UP	30	8	4	1	5
Gastric or duodenal ulcers (n = 84)	SA	24	24	10	16	10
	AGE	21	18	12	15	18
	UP	7	3	6	3	65

*Histological diagnosis using the criteria of Whitehead *et al* ⁶

†Culture or silver stain.

Table 2 Distribution of IgA antibody responses determined using three *C. pylori* antigen preparations

Clinical group	Antigen preparations	No of samples giving absorbance values (× 1000)				
		< 250	< 500	< 750	< 1000	> 1000
Gastritis* and <i>C. pylori</i> detected† (n = 67)	SA	1	12	24	11	19
	AGE	5	18	18	10	16
	UP	0	1	2	7	57
No gastritis and <i>C. pylori</i> not detected (n = 48)	SA	7	26	13	2	0
	AGE	18	19	9	0	2
	UP	7	20	12	4	5
Gastric or duodenal ulcers (n = 84)	SA	3	20	25	17	19
	AGE	8	23	26	16	11
	UP	4	4	4	9	63

*Histological diagnosis using the criteria of Whitehead *et al* ⁶

†Culture or silver stain.

Table 3 Percentage specificity, sensitivity, and predictive values for three *C. pylori* antigen preparations

Characteristic	Immunoglobulin class	Surface antigen	Acid glycine extract	Urease preparation
Specificity	IgG	92	94	90
	IgA	69	77	90
Sensitivity	IgG	82	82	97
	IgA	81	66	85
Predictive value of a true positive	IgG	93	93	93
	IgA	78	80	92
Predictive value of a true negative	IgG	79	79	96
	IgA	70	62	81

≥ 1000 for UP. The percentage of patients with gastritis, *C. pylori*, and raised titres was 81% for SA, 66% for AGE, and 85% for UP. In contrast, the percentage values for patients without gastritis, no *C. pylori*, but with raised titres was 31% for SA, 23% for AGE, and 10% for UP. For patients with gastric or duodenal ulcers significantly raised titres were 73% with SA, 63% with AGE and 75% with UP.

Specificity, sensitivity, and predictive values of the three antigen preparations are presented in table 3. The AGE antigen preparation showed the greatest specificity (94%) in the IgG assay; the UP was the most specific in the IgA assay (90%). The UP showed the greatest sensitivity in both the IgG (97%) and the IgA

(85%) assays. Overall, all three antigen preparations showed greater specificity and sensitivity in the IgG assay than in the IgA assay.

All three antigen preparations gave an identical predictive value of 93% for a true positive result in the IgG assay but the UP was the most predictive in the IgA assay (92%). The UP was also the most predictive of a true negative in the IgG assay (97%) and the IgA assay (81%).

The combinations of the results with the three antigen preparations in the IgG assay are shown in table 4. Of those patients with gastritis and organisms detected, four had raised IgG antibody titres to a single antigen preparation alone and one patient was

Table 4 IgG antibody to *C pylori* detected using three antigen preparations

Results with antigen preparations			No of samples with raised IgG antibody detected in each clinical group		
Surface antigen (≥ 500)*	Acid-glycine extract (≥ 500)	Urease preparation (≥ 1500)	Gastritis <i>C pylori</i> detected (n=67)	No gastritis <i>C pylori</i> not detected (n=48)	Patients with gastric or duodenal ulcers (n=84)
+	+	+	47	2	49
+	+	-	0	0	6
+	-	+	8	2	3
+	-	-	0	0	2
-	+	+	7	1	7
-	+	-	1	0	1
-	-	+	3	0	3
-	-	-	1	43	13

*Absorbance value indicating significantly raised antibody titres.

negative with all of the antigen preparations. Furthermore, this patient did not show raised IgA antibody titres to any of the antigen preparations. Five patients without gastritis and no *C pylori* had raised antibody titre to two or more antigen preparations. Of these five patients, three showed evidence of raised IgA antibody titres (two showed raised IgG antibody with all three antigen preparations and one showed raised IgG antibody with SA and UP). Six of the 84 patients with ulcers showed raised titres to a single antigen preparation and 13 were negative.

Discussion

Antigen preparations used for serodiagnosis or epidemiological surveys of *C pylori* associated gastritis should have high levels of specificity and sensitivity. The three antigen preparations evaluated in this study are relatively crude preparations and contain multiple protein bands when examined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (results not shown). The acid glycine extract (AGE) prepared in the present study produced an almost identical protein profile as similar extracts prepared by other workers.^{2,3} Moreover, the protein profile of the surface antigen (SA) was very similar to that obtained with the AGE, indicating that similar antigens are present in both preparations. Although the SA and AGE are crude preparations, they gave similar levels of specificity (92 and 94%) and sensitivity (82%) in the IgG ELISA. Either of these two preparations, therefore, could be used in an ELISA but the SA is easier to prepare. The urease preparation was the most sensitive (97%) and was slightly less specific (90%) than the other two antigen preparations. SDS-PAGE analysis of this preparation has shown that it contains many of the protein bands found in the SA and AGE, plus additional bands, and is rich in urease. It is therefore difficult to state which of the proteins are acting as antigens in the ELISA and therefore further Western blot studies are necessary to

elucidate the role of the cellular proteins and urease proteins present in this preparation. This crude urease preparation is, however, a useful starting point for purification of the urease antigen of *C pylori* and as this is an important antigen it is likely to be used in the development of future ELISA tests.

Problems of specificity are most likely to occur with preparations containing proteins shared by other organisms, in particular *C jejuni*. The flagellar proteins of *C pylori* are known to cross react with antibodies produced against *C jejuni*.² The SA and AGE preparations contained less of these proteins than the UP and this may explain the difference in specificity between these antigens. In a previous study Dent *et al* reported that their urease preparation had a specificity of 79%,⁴ but in our assay this preparation gave an improved specificity of 90%. This may be due to the use of a higher dilution of test sera in our study and hence a diluting out effect of cross reacting antibody.

Other factors can also contribute to the determination of the specificity of the results obtained, the most important being the technique used to decide if a patient has a gastritis and *C pylori* present in the stomach. In the present survey this was done by examining biopsy specimens. Poor quality of specimens, presence of intestinal metaplasia, and patchy distribution of both organisms and gastritis can lead to an underestimation of the true incidence of infection. In our study we found five patients who seemed to give positive serological reactions in the IgG assay. Two of these patients were probably incorrectly classified as giving false positive results because both showed raised IgG and IgA antibody titres to all three antigen preparations; one was designated as having duodenal ulcer and the other patient had a gastric ulcer. Only a single "antral" biopsy specimen was collected from both patients. If these two patients were treated as showing true positive results then the specificity and predictive value of the ELISA tests with all three antigen preparations would be enhanced.

The three other patients probably gave genuine false

positive results because there was no histological or clinical evidence to contradict the serological results, although one of these patients had raised IgG and IgA antibodies to *C pylori*.

The urease preparation was the most sensitive (97%), gave the highest predictive values, and had a probable specificity of 93% in the IgG-ELISA. It is therefore the antigen preparation of choice for ELISA tests, and our studies suggest that estimation of the IgG titre alone is necessary for screening patients attending endoscopy clinics.

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