The prevalence of anti-*Helicobacter (Campylobacter) pylori* antibodies in patients and healthy blood donors

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Summary. An enzyme linked immunosorbent assay (ELISA) with a sonicated suspension of *Helicobacter (Campylobacter) pylori* as antigen was used to detect anti-*H. pylori* antibodies in 517 patients without dyspepsia or peptic ulcer symptoms and 401 healthy blood donors. The criterion of seropositivity was determined from a receiver operating curve computed with the values of optical densities of 48 sera from dyspeptic patients with proven helicobacter-associated gastritis and 16 sera from dyspeptic patients with proven helicobacter-associated gastritis and 16 sera from dyspeptic patients with normal antral mucosa and no microbiological or histological evidence of *H. pylori* infection. The 227 (44%) seropositive persons amongst the patient group appeared to be significantly higher than the 142 (35%) sera with antibodies in the blood donors tested ($p < 0.03$), even when adjustment was made for increasing age. We conclude that the prevalence of antibodies against *H. pylori* increases with age and that although antibodies are more prevalent in patients attending a hospital than in healthy blood donors, seropositivity suggestive of current or past infection can be found in one third of a randomly chosen population of blood donors.

Introduction

Helicobacter (Campylobacter) pylori has been implicated as a common cause of chronic active gastritis and, possibly, peptic ulcer disease.^{1–5} Early debate as to whether *H. pylori* is an opportunist pathogen attracted to inflamed antral mucosa, or is implicated as the cause of type B (antral) gastritis, tended to be resolving in favour of the latter hypothesis.² Earlier studies on the prevalence of gastritis have shown an increase with age.^{6–8} Since type B (antral) gastritis is almost always associated with *H. pylori*^{1–5} an increasing prevalence of this micro-organism with age would be expected. However, because diagnosis of active *H. pylori* infection has previously relied on invasive diagnostic procedures (endoscopy and biopsy), large scale population studies are sparse and no exact data are available on the presence of this infection in relation to age.

Patients infected with *H. pylori* develop a local as well as a systemic antibody response,^{9–11} and serological techniques can be used for the assess-

ment of this response.^{12,13} In a previous study we demonstrated that the presence of antibodies in a population of dyspeptic patients could be correlated with the presence of helicobacter-associated gastritis.¹⁴ Therefore, it would appear that detection of antibodies to *H. pylori* could be used for epidemiological studies in larger population groups without the necessity of upper gastrointestinal endoscopy. This would enable the prevalence of antibodies in healthy individuals and acute or chronically ill patients who did not have dyspepsia, to be assessed, since at present there are only limited data available on healthy persons.^{15,16}

In this paper we report the results of a serological study with an ELISA assay, performed to assess the prevalence of antibodies against *H. pylori* in healthy blood donors and non-dyspeptic patients in relation to age.

Materials and methods

Sources of sera

Blood (serum) samples were obtained from two groups of individuals: (1), 517 patients—233 men and 284

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women, mean age 56 (range 2–89) years, comprising a heterogenous group of in- and out-patients who were not referred because of dyspeptic complaints nor underwent endoscopy for follow-up of peptic ulcer disease or other reasons related to the upper abdominal tract; (2), 401 healthy blood donors—362 men and 39 women, mean age 42 (range 19–65) years.

As a reference population, serum samples obtained from 64 patients (35 men, 29 women, mean age 64, range 17–85 years) referred for endoscopy because of dyspepsia were used. Of these, 48 patients were shown to have helicobacter-associated gastritis and 16 had normal antral mucosa on histological examination and *H. pylori* was not detected either microbiologically or histologically.

Serological examination

An enzyme-linked immunosorbent assay (ELISA), modified after the description of Kaldor *et al.*,⁹ was used to measure specific IgG antibodies against *H. pylori*. Four strains of *H. pylori* (grown on blood agar with sheep blood 6%, under microaerophilic conditions) were harvested and killed by heating at 60°C. After sonication and preservation with sodium azide 0.1% a suspension was made and used as bacterial antigen. For pre-coating of flexible polyvinyl chloride microtitration plates (Titertek Immunoassay plate, Flow Laboratories The Netherlands), 100 µl of the suspension was diluted 1 in 50 with 60 mM carbonate buffer (pH 9.6) with sodium azide 0.1%. The coated plates were kept at 4°C for 8–10 h and washed with phosphate-buffered saline with Tween 20 0.5%, pH 7.2 (PBS). After adding bovine serum albumin 1% in PBS and re-incubating for 2 h, the plates were washed again with PBS. For controls buffer solution only was used. The prepared plates were kept in a humid environment at 4°C.

For the test assay 50 µl of patient's serum diluted 1 in 10 in PBS was put into the wells of the test and control plates. After incubation for 90 mins at 37°C, the plates were washed with PBS and 50 µl of peroxidase labelled anti-human immunoglobulin (Peroxidase-conjugated rabbit immunoglobulin to human IgG, γ chain; DAKO) was added. After incubation for a further 90 min, the plates were washed and 50 µl of a colour indicator (σ-phenylenediamine 20 mg with 0.08 µl of H₂O₂ 30%) was added and the plates were incubated at room temperature in the dark. The colour reaction was stopped with 50 µl of 4 M sulphuric acid and the optical density (OD) was read at 492 nm. Control sera and blanks were included in each assay. The measured OD values were normalised with respect to the control references to correct for minor day-to-day variations in the assay.

Cross-reactivity with *Campylobacter jejuni* was assessed by two different methods. Firstly, sera from 21 different patients with microbiologically proven *C. jejuni* infection were tested in the ELISA; mean OD was 0.66 (SD 0.40; only one sample produced an OD of 1.76), well below the cut-off points calculated for the presence of anti-*H. pylori* antibodies as shown below. Secondly, 20 of our positive sera were tested in a specific *C. jejuni* ELISA¹⁷ (courtesy

of W. C. van Dijk, Department of Microbiology, Stichting Samenwerkende Ziekenhuizen Delft, The Netherlands); none gave a positive reaction.

Statistical analysis

Statistical analysis was done with the χ^2 test and the t-test. Sensitivity and specificity values were calculated with standard formula.

Results

Assessment of the cut-off point

For the assessment of the cut-off point in OD values to discriminate between presence or absence of anti-*H. pylori* antibodies, sera from patients of the reference population were used. Sera from the 48 patients with *H. pylori* infection diagnosed histologically or microbiologically, or both, gave a mean OD value of 3.63 (SD 1.24, range 1.06–6.45). The 16 helicobacter-negative patients gave a mean OD value of 0.70 (SD 0.45, range 0.21–1.99). After computing a receiver operating curve (ROC), the optimal values for sensitivity (97.9%) and specificity (94.4%) were found to be at OD 1.5. Therefore, an OD value >1.50 was regarded as indicating the presence of antibodies against *H. pylori* (i.e., a positive result) whereas an OD value <1.50 was interpreted as a negative result. Fig. 1 shows the OD values measured with the ELISA assay in these 64 reference patients.

Antibodies in patients and healthy individuals

The mean OD value for seropositive non-dyspeptic patients was 2.14 (SD 0.67, range 1.51–4.13), and for seropositive blood donors was 2.32 (SD 0.47, range 1.51–3.43). There is no significant difference in OD between the two groups (fig. 2). However, there was a significant difference between the OD values of the seropositive reference population with proven helicobacter-associated gastritis and the seropositive blood donors and non-dyspeptic patients ($p < 0.0001$).

Table I shows the total number of patients and blood donors with antibodies to *H. pylori*; 227 (44%) of the 517 patients gave a positive antibody response, compared with 142 (35%) of 401 normal blood donors ($p < 0.03$). This difference remained significant after correction for age and sex.

Antibodies in different age cohorts of non-dyspeptic patients and healthy blood donors

In both groups, a parallel rise in the number of

Fig. 1. OD values for sera of the reference population. HP +, sera from patients with microbiological or histological evidence of *H. pylori* infection; HP -, patients with no such evidence. The dotted line indicates the cut-off point at OD 1.50 as calculated from the Receiver Operating Curve.

positive sera was found with increasing age. Table II shows the numbers of positive and negative sera in each age cohort.

Discussion

The presence of antibodies against *H. pylori* has been described previously in patients with histologically confirmed type B (antral) gastritis.^{16,18,19} Therefore, serological studies have been used to determine the prevalence of gastritis in larger population groups.^{15,20} However, whether detection of a systemic response to the micro-organism implies the presence of active helicobacter-associated gastritis remains questionable. Cross-reactivity with antibodies against other micro-organisms, notably *C. jejuni*, might affect the results of an assay used to detect anti-*H. pylori* antibodies. However, in a previous study we demonstrated that, in our

Fig. 2. Mean OD values and SD of sera from seropositive and seronegative non-dyspeptic patients and healthy blood donors; □, positive sera; ▨, negative sera.

ELISA, there was no such interference with sera from patients with proven *C. jejuni* infection.¹⁵ Alternatively, anti-*H. pylori* antibodies could persist long after exposure to the micro-organism and remain detectable after the initially invoked gastritis has subsided. Other studies have shown decreasing IgG antibody titres after the successful treatment of helicobacter-associated gastritis.^{21,22}

The results obtained with the reference population previously reported¹⁴ indicated that the presence of a systemic antibody response in dyspeptic patients was closely related to active helicobacter-associated gastritis. This is also evident from the high sensitivity and specificity values found after computing an ROC at OD 1.50 in the reference population in this study. These data suggest that the presence of antibodies may reflect active *H. pylori* infection. However, these conclusions cannot be extrapolated from our present results because the OD found in the reference population with helicobacter-associated gastritis is significantly higher than that found in the seropositive non-dyspeptic patients and normal blood donors. Since the antibody concentration decreases after eradication or suppression of *H. pylori*, our findings could indicate present or past infection in these individuals. To determine how many members of our two groups had active *H. pylori* infection would require microbiological and histological confirma-

Table I. Occurrence of positive and negative sera in non-dyspeptic patients and normal blood donors

Source of sera	Number tested	Number of sera with OD values	
		≥ 1.5 (positive)	< 1.5 (negative)
<i>Total</i>			
Non-dyspeptic patients	517	227 (44%)	290 (56%)
Blood donors	401	142 (35%)	259 (65%)
		p < 0.03	
<i>Age and sex adjusted</i>			
Non-dyspeptic patients	381	162 (43%)	219 (57%)
Blood donors	401	142 (35%)	259 (65%)
		p < 0.05	

tion of the presence of helicobacter-associated gastritis.

Our data show that antibodies against *H. pylori* can be detected more frequently in non-dyspeptic patients than in blood donors. Although both groups are not fully comparable, this finding remains after adjustment for age and sex, implying that the prevalence of active gastritis is or was higher in acutely or chronically ill patients. In an earlier study²³ a high prevalence of non-ulcer dyspepsia and gastritis in patients admitted to hospital was observed.²³ The explanation for this phenomenon can only be speculative. In a previous study we found *H. pylori* in a large number of antral biopsy specimens from dyspeptic patients with normal antral mucosa, although the micro-organisms were present in low numbers.²⁴ Therefore we suggested that there is a balance between virulence of *H. pylori* and local mucosal defence

mechanisms, which when distorted would lead to gastritis. It is tempting to assume that in patients, who are generally in a suboptimal condition, this equilibrium is liable to be distorted, resulting in induction of gastritis.

In keeping with data from others^{11, 25-27} we found an increase in occurrence of antibody with increasing age, in patients as well as in donors. The increasing prevalence of antibodies suggests that the micro-organism is acquired later in life, posing the question of how it is acquired. In a study of mentally retarded patients in an institution,¹⁵ the percentage of individuals with antibodies was significantly higher than in age-matched healthy controls, and correlated with the duration of stay in the institution. This suggests person to person transmission.

The most impressive observation from our study is that approximately one third of a randomly chosen population of blood donors appear to have significant levels of antibodies against *H. pylori* and thus an unknown number of these individuals will have active helicobacter-associated gastritis. The debate continues about the relationship between gastritis and dyspeptic complaints.^{6, 28, 29} Although our study design did not include data on dyspeptic complaints in the blood donors tested, it may be assumed that in general these "healthy" individuals did not suffer from dyspeptic complaints to a considerable extent. This raises the question of the clinical relevance of *H. pylori* infection. A definitive answer to this issue will require further studies evaluating evidence of *H. pylori* infection in relation to standard computerised questionnaires on dyspeptic complaints. Such a study is currently in progress.

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Table II. Occurrence of sera positive and negative for antibodies against *H. pylori* in consecutive age cohorts of non-dyspeptic patients and blood donors

Age cohort	Number of sera from			
	non-dyspeptic patients		blood donors	
	positive	negative	positive	negative
0-10	1	5	—	—
11-20	2	10	0	5
21-30	18	39	10	43
31-40	13	37	35	92
41-50	27	37	54	82
51-60	43	46	36	32
61-70	61	62	7	5
71-80	46	36	—	—
81-90	16	18	—	—

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