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Evaluation of Western Blot CagA Seropositivity in *Helicobacter pylori*-Seropositive and -Seronegative Subjects

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CagA seropositivity is an important risk factor for gastric adenocarcinoma and duodenal ulcer. However, CagA seropositivity is also found in *Helicobacter pylori*-seronegative subjects. Is CagA seropositivity in these subjects a sign of a past *H. pylori* infection, or does it represent a false-positive reaction? This study investigates the intensity of the CagA immune reaction and the variation in CagA seroprevalence with year of birth for 650 subjects belonging to the Malmö Preventive Medicine cohort. CagA and *H. pylori* seroprevalences were determined by Western blot analysis (Helicoblot 2.1) and enzyme-linked immunosorbent assay. The peak intensity of the CagA band was significantly lower in *H. pylori*-seronegative subjects than in those with concomitant *H. pylori* seropositivity. In *H. pylori*-seropositive subjects, peak CagA intensity had a bimodal distribution. The prevalence of CagA-seropositive but *H. pylori*-seronegative subjects increased successively and significantly with year of birth, in contrast to the prevalence of CagA-seropositive and *H. pylori*-seropositive subjects, which decreased significantly. However, within *H. pylori*-seropositive and -seronegative subgroups, CagA seroprevalences were constant for different birth cohorts. If CagA seropositivity in *H. pylori*-seronegative subjects represents a past *H. pylori* infection, there must have been some mechanisms of eradication that were more common in younger subjects and that were of more importance than the presence of gastric atrophy and the longer duration and higher prevalence of *H. pylori* infection found in older subjects. Antibiotic treatment of *H. pylori* was not common practice at the time of enrollment. On the other hand, a false-positive reaction would be constant and independent of birth cohorts, as with the *H. pylori*-seronegative subgroup of our study. Peak CagA intensity in *H. pylori*-seronegative subjects corresponded to the lower part of the bimodal distribution of peak CagA intensity in *H. pylori*-seropositive subjects. We conclude that a major proportion of CagA seropositivity in *H. pylori*-seronegative subjects represents a false-positive reaction. Peak CagA intensity has a bimodal distribution in *H. pylori*-seropositive subjects. Low-intensity CagA seropositivity in *H. pylori*-seropositive subjects is indeterminate, representing both false-positive and true-positive reactions.

The *Helicobacter pylori* virulence factor *cagA* (cytotoxin-associated gene A) and its highly immunogenic protein product CagA are important risk factors for the development of gastric adenocarcinoma and duodenal ulcer (1, 2, 7, 9, 10, 13). Serological tests may be useful in predicting the risk of developing these diseases. Recently, CagA seropositivity has been found in subjects seronegative for the bacterium itself (4, 5). It is hypothesized that CagA seropositivity in *H. pylori*-seronegative subjects may either be a sign of a past *H. pylori* infection (3) or represent a false-positive reaction that may be due to a nonimmune protein-protein interaction or due to cross-reactivity.

The objective of this study was to investigate whether CagA seropositivity in *H. pylori*-seronegative subjects determined with a Western blot method represents a false-positive immune reaction or a sign of a past infection. The strength of a false-positive reaction to the test antigen may differ from that of a true-positive reaction. Also, a sign of a past infection would be expected to be more prevalent among older subjects because of the longer duration and the higher prevalence of *H. pylori* infection in older individuals and because of the spontaneous eradication of *H. pylori* associated with gastric atrophy. On the other hand, if CagA seropositivity in *H. pylori*-seronegative subjects represents a false-positive reaction, CagA-seropositive and *H. pylori*-seronegative subjects would be expected to be less prevalent in older individuals because of the higher prevalence of *H. pylori* infection in these individuals.

This study investigated the intensity and the change in seroprevalence over time of the 116-kDa CagA band in *H. pylori*-seropositive and -seronegative subjects as detected by the Helicoblot 2.1 test (Genelabs Diagnostics, Singapore) and was performed with the population-based serum bank of the Malmö Preventive Medicine cohort at Malmö University Hospital, Malmö, Sweden.

MATERIALS AND METHODS

Study subjects. Selected samples from the Malmö Preventive Medicine health-screening cohort were used. The 650 samples used had been selected as controls matched to patients with gastric or esophageal malignancies in a case-control study performed by our group. The matching criteria were gender, date of birth (±6 months), and date of enrollment (±6 months). In order to make the selected material representative of the Malmö Preventive Medicine cohort, a set of correction factors were used. Each cell consisted of subjects born in the same year and of the same sex. The quotient of the relative cell size of the Malmö Preventive Medicine cohort and the relative cell size of the study group was used as a correction factor. Corrected sums of numbers of seropositive subjects and numbers of individuals in cells were estimated, and the quotients of these sums were used to estimate accurate seroprevalences. The Preventive Medicine cohort consists of 32,906 middle-aged Malmö citizens invited to a health-screening

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TABLE 1. Matching criteria of the 650 study subjects

Parameter	Study subjects[a]	Malmö Preventive Medicine cohort
Mean yr of birth (±SD)	1931 (±6.1)	1934 (±6.3)
Mean yr of enrollment (±SD)	1981 (±3.0)	1982 (±3.5)
% of males	85	70

[a] Study subjects were originally chosen as matched control subjects of a case-control study of esophagogastric cancer. The male sex and older individuals were slightly overrepresented among the study subjects compared to members of the Malmö Preventive Medicine cohort.

investigation (23,104 men and 9,802 women) who were enrolled from 1974 to 1992. All citizens in the city of Malmö belonging to the male birth cohorts corresponding to the years 1921, 1926 to 1942, 1944, 1946, and 1948 (termed, e.g., cohort 1921) and to the female birth cohorts 1928, 1930–1936, 1938, and 1941 were invited; the mean participant rate was 75%, ranging from 62 to 85% in different birth cohorts. At enrollment, plasma and serum samples from each participant were frozen at −20°C.

Western blot analysis. CagA seropositivity and *H. pylori* seropositivity were estimated using the commercial Western blot assay Helicoblot 2.1 (Genelabs Diagnostics). Helicoblot 2.1 has a reported sensitivity of 96% and specificity of 95% compared to histology, culture, the rapid urease test, or the urea breath test (manufacturer data). Added onto the immunoblot strip, Helicoblot 2.1 has a separate current infection marker consisting of a recombinant antigen with a positive predictive value of 85 to 94%. Reactive and nonreactive control sera were included in each test kit together with a photocopy of the results for the positive reactive control. The molecular weights of those bands needed for *H. pylori* seropositivity determination were indicated on this photocopy. The bacterial lysate and the reactive positive controls of all kits belonged to a single batch (Matthew Maks, Genelabs Diagnostics, personal communication). Helicoblot strips were incubated with sera diluted 1:100 for 1 h at room temperature and then incubated with goat anti-human immunoglobulin G (IgG) conjugated with alkaline phosphatase included in the kit for 1 h at room temperature. The strips were then developed with 5-bromo-4-chloro-2-indolyl-phosphate and nitroblue tetrazolium for 15 min. The strips were scanned (model GS-700 densitometer; Bio-Rad Laboratories, Hercules, Calif.) at a resolution of 600 dots per inch. The band analysis computer program Quantify One (Bio-Rad Laboratories), which contained tools for magnification, contrast enhancement, and molecular weight determination, was used to aid manual identification of bands. Identification of a band was based on the shape of an area with increased intensity. This area was examined at different levels of intensity from its maximum intensity to the lowest intensity level at which the area reached across the strip. A band had to reach across the strip, and the shape of the increased intensity had to have a more prominent extension across the strip than along the strip. A positive current infection marker had to have a detectable increase in intensity over at least half of the rectangular current infection marker area and have well-demarcated edges. The photocopy of the results for the positive reactive control defined molecular weights. Molecular weights for bands in the sample strips were suggested by the Quantify One program and manually verified or adjusted according to the band pattern of the strip. The peak intensity of *H. pylori*-specific bands was determined after subtraction of background intensity and adjustment by the corresponding band intensity of the positive reactive control belonging to each test kit. The criteria for *H. pylori* seropositivity according to the Helicoblot test were as recommended by the manufacturer: the presence of the 116-kDa CagA band in combination with the current infection marker, the combination of the 19.5- and 30-kDa bands, or at least one of the 89-, 37-, and 35-kDa bands.

IgG ELISA. *H. pylori* seropositivity according to an IgG enzyme-linked immunosorbent assay (ELISA) was determined through an in-house method. Antigen was prepared using ultrasonification of *H. pylori* colonies from the reference strain CCUG 17874 (6). The absorbance values have previously been shown to be distributed as two well-defined Gaussian curves with only a small overlap (11), with the cutoff (absorbance, 0.700) in between these Gaussian peaks. The sensitivity and specificity have been reported to be 0.98 and 0.81, respectively. In that report, culture from antrum was used as the "gold standard" (6).

Statistical methods. The Wilcoxon two-sample test was used for testing the difference between continuous parameters. Multivariate linear regression models from the computer program SAS (version 6.12; SAS Institute Inc., Cary, N.C.) were used to determine changes in prevalence with time. A P value of <0.05 was regarded as significant; two-sided tests were used.

TABLE 2. Birth cohort distribution, gender, and age of the 650 included study subjects[a]

Birth cohort	No. of males	No. of females	Mean age (yr)	SD
1921	76	0	59.6	0.5
1926–28	143	16	53.4	3.0
1929–33	180	31	50.6	3.5
1934–38	88	40	45.7	4.4
1939–43	44	8	40.3	3.0
1944–48	24	0	34.1	1.2
Total	555	95		

[a] The mean age at enrollment in each birth cohort corresponded to a mean year of enrollment of 1981 (SD, ±3.0).

RESULTS

Study population. The 650 study subjects were originally chosen as matched control subjects of a case-control study of esophagogastric cancer. Males and older individuals were slightly overrepresented among the study subjects of our group compared to the subjects in the Malmö Preventive Medicine cohort (Tables 1 and 2). Correction factors could be estimated for all sex and birth year cells. The mean age at enrollment corresponded to a mean year of enrollment of 1981 (standard deviation, ±3.0).

CagA seroprevalence. CagA seropositivity was found in 346 of 376 subjects (92%) who were *H. pylori* seropositive by the Helicoblot 2.1 test and in 123 of 274 subjects (45%) who were *H. pylori* seronegative by that test (Table 3). Similar CagA seroprevalences were found after adjustment so that our study subjects would be representative of the Malmö Preventive Medicine cohort: 298 of 327 (91%) *H. pylori*-seropositive subjects and 135 of 323 (42%) *H. pylori*-seronegative subjects (Table 3).

Peak CagA intensity. The peak intensity of the CagA band ranged from 0 to 215 arbitrary units (Fig. 1). Visibility to the naked eye corresponded to a peak intensity of about 10 arbitrary units. The median peak CagA band intensity in subjects

TABLE 3. CagA seropositivity prevalence in *H. pylori*-seropositive and -seronegative subjects[a]

Subjects[b]	No. (%) of subjects	
	Crude	Adjusted to MPM
H. pylori-seropositive		
High-intensity CagA band	305 (81)	260 (80)
Low-intensity CagA band	41 (11)	38 (12)
CagA seronegative	30 (8)	29 (9)
Total	376 (100)	327 (100)
H. pylori-seronegative		
High-intensity CagA band	27 (10)	26 (8)
Low-intensity CagA band	96 (35)	109 (34)
CagA seronegative	151 (55)	188 (58)
Total	274 (100)	323 (100)

[a] CagA seropositivity is shown as the presence of a high- or low-intensity CagA band, depending on whether the peak intensity of the CagA band is above or below 50 arbitrary intensity units. Seroprevalences are given as crude figures and as figures adjusted so that they are representative of the Malmö Preventive Medicine cohort (MPM).

[b] *H. pylori* serostatus estimated with the Helicoblot 2.1 test.

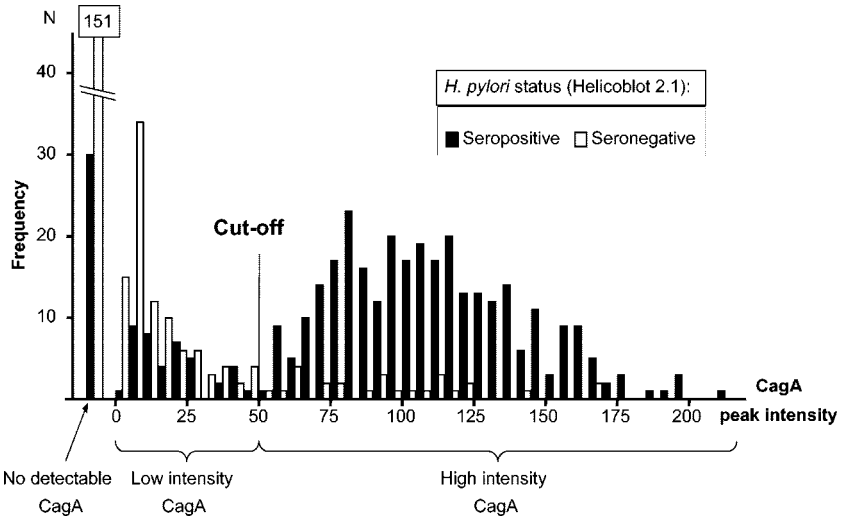

FIG. 1. The peak intensity of the CagA band was higher in *H. pylori*-seropositive subjects than in *H. pylori*-seronegative subjects. No CagA band could be detected in the sera of 151 subjects. There was a bimodal distribution of peak CagA intensity in *H. pylori*-seropositive subjects.

who were *H. pylori* seropositive by the Helicoblot 2.1 test and CagA seropositive was 101.9 (quartiles, 77.2 to 126.4) arbitrary intensity units compared with 15.0 (quartiles, 7.3 to 44.5) arbitrary intensity units in *H. pylori*-seronegative but CagA-seropositive subjects, the difference being highly significant ($P \ll 0.0001$). The peak CagA intensity distribution diagram verified that *H. pylori*-seropositive and -seronegative subjects had different distributions of peak CagA intensity (Fig. 1). The peak CagA intensity distribution diagram also revealed a bimodal peak CagA intensity distribution in *H. pylori*-seropositive subjects. A cutoff positioned in between the two peaks of the *H. pylori*-seropositive bimodal CagA intensity distribution at an intensity level of 50 arbitrary units categorized the peak CagA intensities into a high-intensity CagA group and a low-intensity CagA group. Correction for sex and birth year did not affect the peak CagA intensity distribution diagram (data not shown). Specifically, the difference in peak CagA band intensity between *H. pylori*-seropositive and -seronegative subjects remained, as well as the bimodal distribution among *H. pylori*-seropositive subjects. There was no correlation between CagA intensity and freezing time ($r = 0.0011$).

Dependence between *H. pylori* seropositivity and CagA seropositivity. In 330 of 376 (88%; adjusted to 280 of 327 or 86%) *H. pylori*-seropositive cases, more than one of the criteria for *H. pylori* seropositivity was fulfilled. CagA seropositivity in combination with the separate infection marker was the only criterion for *H. pylori* seropositivity in 11 of 376 (2.9%; adjusted to 12 of 327 or 3.7%) cases. Among 137 (adjusted to 148) subjects with low-intensity CagA seropositivity, 7 of 41 (17%; adjusted to 10 of 38 or 26%) were classified as *H. pylori* seropositive solely on the criteria of CagA positivity and the presence of the current infection marker.

Low-intensity CagA bands in *H. pylori*-seropositive and -seronegative subjects. Only 41 of 346 (12%; adjusted to 38 of 298 or 13%) CagA- and *H. pylori*-seropositive subjects were CagA seropositive at a low intensity (Table 3). In contrast, 96 of 123 (78%; adjusted to 109 of 135 or 81%) *H. pylori*-seronegative but CagA-seropositive subjects were CagA seropositive at a low intensity (Table 3).

***H. pylori* and CagA seroprevalences over time.** Helicoblot 2.1 *H. pylori* seroprevalence decreased 1.37% (standard error [SE], ±0.32; $P = 0.0001$) with each increasing year of birth. IgG ELISA *H. pylori* seroprevalence decreased as well by 1.14% (SE, ±0.32; $P = 0.0005$) per year of birth (Fig. 2) (Table 4). The prevalence of subjects that were *H. pylori* seropositive according to the Helicoblot 2.1 test and had a high- or a low-intensity CagA band decreased with each increasing year of birth by 1.06% (SE, ±0.32; $P = 0.0012$) or 0.35% (SE, ±0.16; $P = 0.029$), respectively. In contrast, there was an increase with year of birth in the prevalence of subjects that were *H. pylori* seronegative according to the Helicoblot 2.1 test but had a low-intensity CagA band: 0.54% (SE, ±0.23; $P = 0.021$) per year (Fig. 2) (Table 4). The prevalence of *H. pylori*-seronegative subjects with a high-intensity CagA band did not change with birth date; the rate of increase per year of birth was 0.18% (SE ±0.13; $P = 0.17$) (Fig. 2) (Table 4). Within the *H. pylori*-seropositive and -seronegative groups, there were constant proportions at different birth years of both high- and low-intensity CagA-seropositive subjects (Fig. 3). The changes with increasing year of birth of the proportions of high- and low-intensity CagA bands among *H. pylori*-seropositive subjects were not significant: 0.10% (SE, ±0.35; $P = 0.78$) and 0.38% (SE, ±0.28; $P = 0.18$), respectively (Fig. 3). In the *H. pylori*-seronegative group, neither of the changes with increasing year of birth of the proportions of high- and low-intensity CagA were significant: 0.09% (SE, ±0.30; $P = 0.29$) and 0.14% (SE, ±0.48; $P = 0.77$), respectively. All linear regression analyses were adjusted for sex.

DISCUSSION

This study shows that the prevalence of subjects that were *H. pylori* seronegative but CagA seropositive by the Helicoblot 2.1 test was lower in older individuals and increased succes-

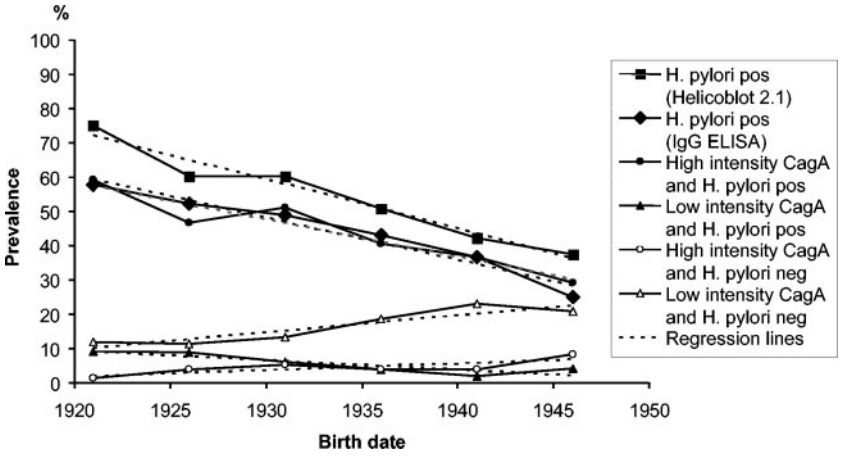

FIG. 2. The prevalences of different serological markers of *H. pylori* infections are plotted against the years of birth of 650 subjects. The prevalences of *H. pylori* seropositivity determined by the Helicoblot 2.1 test and an IgG ELISA decreased with increasing year of birth. The prevalences of *H. pylori*-seropositive subjects with high- or low-intensity CagA seropositivity as determined by the Helicoblot 2.1 test decreased as well with increasing year of birth. In contrast, the prevalences determined by Helicoblot 2.1 testing of *H. pylori*-seronegative subjects with high- or low-intensity CagA seropositivity increased with year of birth. pos, positive; neg, negative.

sively with year of birth, in contrast to a successive decrease in prevalence of subjects that were both *H. pylori* and CagA seropositive. The proportions of CagA-seropositive subjects within *H. pylori*-seropositive and -seronegative subgroups were constant in different birth cohorts. Furthermore, the study shows that the median peak CagA intensity was significantly lower in *H. pylori*-seronegative subjects than in *H. pylori*-seropositive subjects and that peak CagA intensity had a bimodal distribution in *H. pylori*-seropositive subjects.

Almost half of the subjects who were *H. pylori* seronegative by the Helicoblot 2.1 test were CagA seropositive. The Helicoblot 2.1 test has a reported sensitivity of 96%. Thus, most *H. pylori*-seronegative but CagA-seropositive subjects were not infected with *H. pylori* at the time of blood sampling. Could CagA seropositivity in an *H. pylori*-seronegative subject be a sign of a past *H. pylori* infection? In our material, the proportion of CagA seropositivity in the *H. pylori*-seronegative subgroup was constantly about 42% in different birth cohorts. Similarly, in the *H. pylori*-seropositive group, the proportion of CagA seropositivity was constantly about 91%. Because the prevalence of *H. pylori* decreased with increasing year of birth, the prevalence of CagA-seropositive but *H. pylori*-seronegative subjects was highest in the youngest birth cohorts (Table 4).

If CagA seropositivity in subjects who were *H. pylori* seronegative by the Helicoblot 2.1 test represents a past *H. pylori* infection, there must have been some mechanism of eradication that was more common in younger individuals and that was of more importance than the presence of gastric atrophy and the longer duration and higher prevalence of *H. pylori* infection found in older individuals. *H. pylori* eradication was not a generally established therapy at the time when the blood samples of this study were collected. Successively improving living conditions might have increased the spontaneous eradication rate during childhood, leaving CagA seropositivity as a scar of an early infection. Such a mechanism would, though, imply that the *H. pylori* infection rate in childhood is still high in younger individuals. Another possibility might be that antibodies against CagA are retained to a higher degree in younger than in older subjects after an *H. pylori* infection has been resolved. However, the retention time of CagA antibodies af-

TABLE 4. Seroprevalences of *H. pylori* infection in different birth cohorts according to the Helicoblot 2.1 test, ELISA, and CagA seropositivity[a]

Birth cohort	% Seroprevalence of *H. pylori* (95% CI)		% of subjects with indicated peak CagA intensity level (95% CI)			
	Helicoblot positive	ELISA positive	*H. pylori* seropositive		*H. pylori* seronegative	
			High	Low	High	Low
1921	75.0 (65–85)	57.9 (47–69)	59.2 (48–70)	9.2 (3–16)	1.3 (0–4)	11.8 (4–19)
1926–28	60.4 (53–68)	52.2 (44–60)	46.5 (39–54)	8.8 (4–13)	3.8 (1–7)	11.3 (6–16)
1929–33	60.2 (53–67)	48.8 (42–56)	51.2 (44–58)	6.2 (3–9)	5.2 (2–8)	13.3 (9–18)
1934–38	50.8 (42–60)	43.0 (34–52)	40.6 (32–49)	3.9 (0–7)	3.9 (0–7)	18.8 (12–26)
1939–43	42.3 (29–56)	36.5 (23–50)	36.5 (23–50)	1.9 (0–6)	3.8 (0–9)	23.1 (11–35)
1944–48	37.5 (18–57)	25.0 (7–43)	29.2 (11–48)	4.2 (0–12)	8.3 (0–20)	20.8 (4–37)
1934–48	47.1 (40–54)	39.2 (32–46)	38.2 (31–45)	3.4 (1–6)	4.4 (2–7)	20.1 (14–26)
Total	57.8 (54–62)	47.7 (44–52)	46.9 (43–51)	6.3 (4–8)	4.2 (3–6)	14.8 (12–18)

[a] CagA seropositivity was examined for low and high intensity.

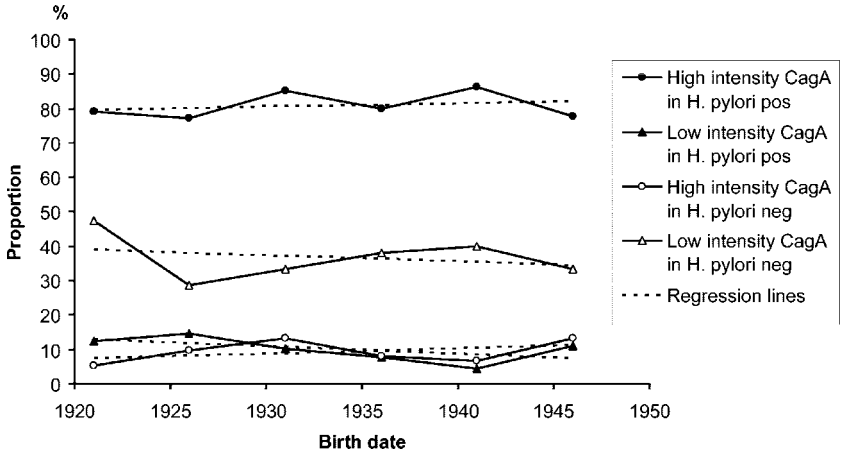

FIG. 3. The proportions of high- and low-intensity CagA bands were constant in different birth cohorts for both *H. pylori*-seropositive and -seronegative subjects. pos, positive; neg, negative.

ter the resolution of *H. pylori* infection would then have to decrease linearly with age and at the specific rate needed to keep the proportion of CagA seropositivity of *H. pylori*-seronegative subjects constant in different birth cohorts, as was found in our material. There was no correlation between peak CagA intensity and freezing time, and there were instances of both increasing and decreasing CagA seroprevalences with year of birth, contradicting the possibility of an effect caused by sample storage.

A more comprehensible explanation of the constant proportion of CagA seropositivity in subjects who were *H. pylori* seronegative by the Helicoblot 2.1 test in different birth cohorts might be that CagA seropositivity in *H. pylori*-seronegative subjects represents a false-positive reaction that may be due to a nonimmune protein-protein interaction or due to cross-reactivity against antibodies formed primarily from a different widely distributed antigen. Such a false-positive reaction would be constant and independent of birth cohorts. However, this kind of false-positive CagA reaction would be uncovered only in *H. pylori*-seronegative subjects. Therefore, with decreasing *H. pylori* seropositivity, this phenomenon would be observed to increase, as was true for our material.

If CagA seropositivity in samples that are *H. pylori* seronegative by the Helicoblot 2.1 test represents a false-positive reaction, it is reasonable to believe that this false-positive reaction occurs also in *H. pylori*-seropositive samples. Among *H. pylori*-seronegative subjects, by cohort-representative values, 42% were CagA seropositive (Table 3). Of these, 81% had a CagA band with a peak intensity below 50 arbitrary intensity units. Among *H. pylori*-seropositive subjects, 29 were CagA seronegative. If the proportion of false-positive reactions to CagA seronegativity in *H. pylori*-seropositive subjects is similar to that in *H. pylori*-seronegative subjects (137 to 188), 21 *H. pylori*-seropositive subjects might have a false-positive CagA reaction, most of whom would have low-intensity CagA seropositivity. Thus, about half of the 38 *H. pylori*-seropositive subjects with low-intensity CagA seropositivity might have had a false-positive reaction. However, only a small fraction of the 260 *H. pylori*-seropositive subjects with high-intensity CagA seropositivity would have had a false-positive reaction. It would therefore be reasonable to consider a low-intensity CagA band in subjects who are *H. pylori* seropositive by the Helicoblot 2.1 test as indeterminate with regard to their CagA status.

Ekström et al. (3) found CagA seropositivity among *H. pylori*-seronegative subjects to be a strong risk factor for noncardia gastric adenocarcinoma in a case-control study with cross-sectional blood sampling. In their study, CagA seropositivity was present among 59 of 79 (75%) *H. pylori*-seronegative gastric cancer cases, in contrast to 10 of 107 (10%) *H. pylori*-seronegative controls. Longitudinal studies have shown that the association between *H. pylori* seropositivity and noncardia gastric adenocarcinoma is stronger when blood sampling is performed more than 10 years before cancer diagnosis (8). It is generally believed that *H. pylori* infections spontaneously disappear because of the gastric atrophy that occurs in the development of intestinal gastric cancer. In an eradication study of ulcer patients with 32 months of follow-up, Sörberg et al. (12) found posttreatment CagA antibody titers to decrease slower than *H. pylori* enzyme immunoassay IgG antibody titers. Thus, there is support for the notion that CagA seropositivity in *H. pylori*-seronegative subjects represents a past *H. pylori* infection when blood sampling is performed close to the time of gastric cancer diagnosis. Whether this is true for the general population is unclear. Fusconi et al. (5) found CagA seropositivity among 8 of 80 (10%) stringently selected subjects who were *H. pylori* negative by five tests (histology, culture, rapid urease test, urea breath test, and IgG ELISA serology). Because of the dissimilarities between CagA and other known bacterial and human polypeptide sequences, Fusconi et al. (5) argue that CagA seropositivity in *H. pylori*-seronegative subjects generally represents a past *H. pylori* infection. In our material, where the Helicoblot 2.1 test was used to determine *H. pylori* and CagA seroprevalences, CagA seropositivity was found among a higher proportion of subjects: 123 of 274 (45%) *H. pylori*-seronegative subjects. We cannot exclude the possibility that a minor proportion of these were infected with *H. pylori* in the past. However, without an explanation of why past infection would be more prevalent in younger individuals, a major proportion of subjects showing CagA seropositivity as

determined by the Helicoblot 2.1 test among *H. pylori*-seronegative subjects would have to represent a false-positive reaction.

Although we classified serostatus for both *H. pylori* and CagA with the same test, only one of five criteria for *H. pylori* seropositivity is based on the CagA serostatus, and then the determination is made in combination with the current infection marker. There was negligible dependence between the two tests (3.7%), except in determining the number of CagA-dependent *H. pylori*-seropositive subjects in the subgroup of individuals seropositive for CagA at a low intensity (26%). This dependence would not interfere with the conclusions of this study. However, we recommend caution in the interpretation of the Helicoblot 2.1 strip when the CagA band is faint and no other criterion for *H. pylori* seropositivity is present.

The Malmö Preventive Medicine cohort is a health-screening cohort of middle-aged Malmö citizens enrolled from 1974 to 1992. The high participation rate makes it a good representative sample of the general middle-aged population. Although adjustment had to be done in order to make the selected study subjects representative of the Malmö Preventive Medicine cohort, the main conclusions of this study were not affected by whether this adjustment was performed or not.

In conclusion, our data suggest that for a major proportion of *H. pylori*-seronegative subjects who are CagA seropositive, the reaction to CagA is a false-positive reaction. Our results also suggest that there is a bimodal distribution of peak CagA intensity in *H. pylori*-seropositive subjects and that the low-intensity proportion of this bimodal peak CagA intensity distribution consists of both true- and false-positive reactions. A low-intensity CagA band among *H. pylori*-seropositive subjects should be regarded as indeterminate concerning CagA status. Further studies will be needed to assess whether these findings are limited to the Helicoblot 2.1 Western blot method or are common properties of Western blot methods measuring CagA seropositivity.

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