Long-term Follow-up Study of Serum Immunoglobulin G and Immunoglobulin A Antibodies After *Helicobacter pylori* Eradication

Seiichi Kato, MD*; Noriko Furuyama, MD‡; Kyoko Ozawa, MD*; Kenji Ohnuma, MD*; and Kazuie Iinuma, MD*

ABSTRACT. *Objective.* **There have been few studies concerning serum titers of anti-*Helicobacter pylori* immunoglobulin G (IgG) antibody >12 months after eradication of the original infection. Moreover, clinical usefulness of immunoglobulin A (IgA) antibody levels remains to be established. The purpose of this study was to investigate long-term responses of serum IgG-specific and IgA-specific antibodies to *H pylori* in children after eradication therapy.**

Study Design. **A total of 34 children, 2 to 17 years of age (mean: 11.7 years) with *H pylori*-associated gastroduodenal disease received eradication therapy (proton pump inhibitor-based dual or triple regimens). Diagnoses included nodular gastritis ($n = 8$), gastric ulcer ($n = 7$), and duodenal ulcer ($n = 19$). Upper gastrointestinal endoscopy and biopsy were performed before the therapy and at 1 to 2 months' posttreatment. *H pylori* infection and eradication were defined by biopsy-based tests; eradication was successful in 28 patients and unsuccessful in 6. Pretreatment IgG was positive in 30 patients (88.2%), and the IgA was positive in 31 (91.2%), who were entered into this study (duration ≤24 months). Serum samples were obtained before treatment and at 1, 3, 6, 12, 18, and 24 months' posttreatment. IgG and IgA antibodies were measured using commercial enzyme immunoassay kits (HM-CAP and PP-CAP; Enteric Products, Inc, New York, NY).**

Results. **Compared with pretreatment values, IgG and IgA antibodies significantly and steadily decreased at 1 through 24 months' posttreatment in successfully treated patients. A decrease in titer of the IgA class was significantly greater than that of the IgG class at 1 to 12 months' follow-up. There was no significant decrease in titer of either antibody in all but 2 patients with eradication failure. A ≥30% decrease in titer of the IgA antibody at 6 months indicated eradication with sensitivity of 90.5% and specificity of 100%. For the IgG antibody, a 30% decrease at 12 months showed equal sensitivity and specificity. Seroreversion rates of IgG and IgA antibodies were 53% and 48% at 12 months and were 86% and 81% at 24 months, respectively. The mean periods from the completion of eradication therapy to seroreversion of IgG and IgA antibodies were 11.2 ± 7.0 and 11.6 ± 7.8 months, respectively (not significantly different). A higher pretreatment titer of IgG antibody was related to a longer period of seroreversion ($r = 0.44$). In one patient, ^{13}C-urea breath test-confirmed reinfection was accompanied by reappearance of significant titers of the IgG and IgA antibodies.**

Conclusions. **A serology test is useful for evaluating eradication in children. Approximately half of patients with successful eradication remained to be IgG-seropositive and IgA-seropositive at 12 months' posttreatment. When a decrease titer in antibody is used for assessing eradication, an endpoint of ≥6 months is required. The IgA antibody may be a more convenient indicator of *H pylori* status than is the IgG antibody. *Pediatrics* 1999; 104(2). URL: http://www.pediatrics.org/cgi/content/full/104/2/e22; *Helicobacter pylori, serum antibody, eradication, IgG, IgA.***

ABBREVIATIONS. IgG, immunoglobulin G; EIA, enzyme immunoassay; IgA, immunoglobulin A; EV, enzyme immunoassay value.

Helicobacter pylori is recognized now as an important etiologic factor in peptic ulcer disease and chronic gastritis.[1,2] The National Institutes of Health has recommended eradication therapy for all ulcer patients with *H pylori* infection.[3] Successful eradication of the organism usually signifies a permanent cure of peptic ulcer disease.

Biopsy-based tests, including culture, histology, or urease tests, are essential for diagnosis of *H pylori* infection and for assessment of eradication. However, these methods require endoscopy and may show a false-negative result, because the organism can distribute irregularly in the stomach, or because it is suppressed transiently with eradication therapy. On the other hand, noninvasive serology and ^{13}C-urea breath test have the same diagnostic accuracies as biopsy-based tests for detecting *H pylori* infection.[4,5] Because the serum immunoglobulin G (IgG) test has showed high sensitivity and specificity,[6,7] the serology test has been used widely in epidemiologic studies and routine diagnosis of *H pylori* infection. Although most previous serologic studies have used noncommercial enzyme immunoassay (EIA) methods for IgG antibodies to *H pylori*, several commercial EIA kits are now available.[7,8]

Generally, it is thought that *H pylori* infection persists for a lifetime and that the serum IgG antibody level remains high. Although spontaneous elimination of *H pylori* can occur, it is infrequent.[9] Titers of serum IgG antibody decrease significantly ≥12 months after *H pylori* is eradicated in adults[10–14] and in children.[15–17] However, there have been few studies concerning the IgG antibody response after >12

From the *Department of Pediatrics, Tohoku University School of Medicine; and ‡Department of Pediatrics, Sendai City Hospital, Sendai, Japan.
Received for publication Nov 24, 1998; accepted Mar 12, 1999.
Reprint requests to (S.K.) Department of Pediatrics, Tohoku University School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan. E-mail: skato@ped.med.tohoku.ac.jp

months' posteradication.[13–15] Moreover, it is unclear when seroreversion of the IgG antibody is observed. Although the immunoglobulin A (IgA)-specific antibodies to *H pylori* are demonstrated in gastric juice and blood,[18] its clinical usefulness remains to be established.[10,12,16]

The purpose of this study was to investigate the long-term (≤24 months) serologic response of IgG and IgA antibodies in children after eradication therapy. In this quantitative study, the duration from eradication of the organism to seroreversion was also examined.

PATIENTS AND METHODS

Patients

Between March 1995 and December 1997, 34 patients (19 male and 15 female) between 2 and 17 years of age (mean: 11.7 years) with *H pylori*-positive peptic ulcer disease and/or nodular gastritis who received eradication therapy were entered into this study (Table 1). Details of the eradication therapy and clinical course were reported previously.[17,19] Regimens consisted of a proton pump inhibitor (omeprazole or lansoprazole) and one (amoxicillin) or two (amoxicillin plus clarithromycin) antibiotics. The duration of drug administration was 7 or 14 days. All patients underwent upper gastrointestinal endoscopy before eradication therapy and at 1 to 2 months' posttreatment and two or more biopsy specimens were obtained from the gastric antrum. *H pylori* infection and eradication were defined by biopsy-based tests (histology, culture, and urease test). In recent patients, ^{13}C-urea breath test was used together with biopsy-based tests.[20] After 3 mg/kg ^{13}C-urea (CEA Group, Gif-sur-Yvette, France) to a maximum dose of 100 mg was ingested, breath samples were collected at baseline and at 20 minutes. δ $13CO_2$, ≥4.5‰ was considered positive for gastric urease activity. If all posttreatment *H pylori* tests were negative, *H pylori* was considered to have been eradicated. Once eradication was confirmed, patients were followed without any maintenance therapy including antisecretory drugs. The ^{13}C-urea breath test was performed every year after eradication therapy for assessing *H pylori* status. Informed consent for the present study was obtained from all patients and their parents.

TABLE 1. Characteristics of Patients Enrolled in This Study

	Eradication	
	Successful	Unsuccessful
No. of patients (M/F)	28 (16/12)	6 (3/3)
Gastric ulcer	6	1
Duodenal ulcer	16	3
Nodular gastritis	6	2
Age (y)		
Mean ± SD	12.0 ± 3.9	10.3 ± 3.5
Range	2–17	3–14
Pretreatment titer		
No. of positive IgG	24	6
No. of positive IgA	25	6
Follow-up periods (mo)*		
Mean ± SD	15.0 ± 7.7	10.5 ± 4.8
Range	3–24	3–18

* Data of patients in whom pretreatment IgG or IgA was positive.

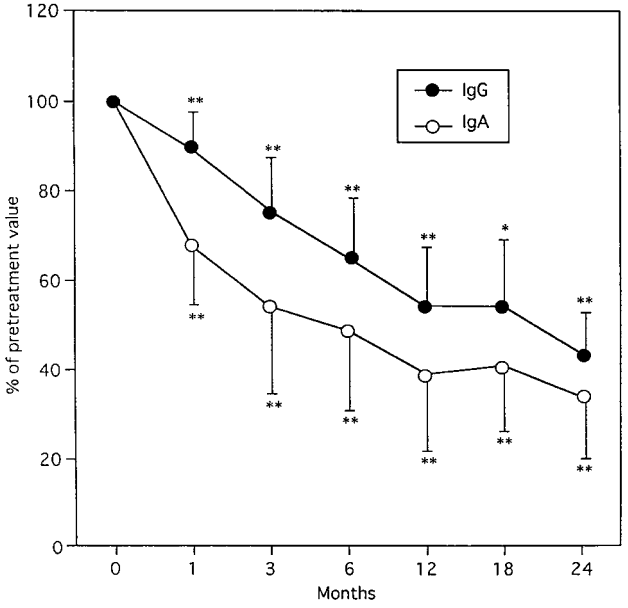

Fig 1. Serum anti-*H pylori* IgG and IgA antibody titers in patients with successful eradication. Posttreatment titers are expressed as percentages of pretreatment titers. Pretreatment versus posttreatment titers (*$P < .01$; **$P < .001$).

Serology

Serum samples were obtained at pretreatment and at 1, 3, 6, 12, 18, and 24 months after the completion of eradication therapy and were frozen at −20°C until assay. Serum titers of IgG and IgA antibodies to *H pylori* were measured using commercial EIA kits (HM-CAP and PP-CAP, Enteric Products, Inc, New York, NY). The HM-CAP kit was designed to detect IgG-specific antibodies against high-molecular-weight cell-associated protein of *H pylori*. The PP-CAP kit was designed to detect IgA-specific antibodies against partially purified cell-associated protein of the organism. All samples were measured together in the same assay, using the same lots of the assay kits for each antibody. As with adults samples, interpretation of the results was as follows: >2.2 EIA value (EV), positive; <1.8 EV, negative; and 1.8 to 2.2 EV, indeterminate for both specific antibodies.

Statistics

The differences between pretreatment and posttreatment antibody titers and the differences between decreases of IgG and IgA antibody titers were analyzed by the paired t test. A χ^2 test was used to examine differences of sensitivity and specificity between titer decreases of IgG and IgA. Analysis of cumulative seroreversion rates was performed by the Kaplan-Meier method. Spearman's correlation coefficient was used to assess a correlation between pretreatment antibody titers and durations to seroreversion in each patient. $P < .05$ was regarded as statistically significant. The values were presented as mean ± SD.

RESULTS

Eradication

Among 34 patients, eradication succeeded in 28 patients and failed in 6. In successfully treated patients, ^{13}C-urea breath tests performed ~12 months after the therapy indicated that all patients studied continued to be free of *H pylori* infection. Of these patients, 1, with a duodenal ulcer, showed a positive ^{13}C-urea breath test at 28 months. In the remaining patients, the ^{13}C-urea breath test was negative during the follow-up periods.

Serology

Before treatment, serum IgG antibody titers were positive in 24 successfully treated patients and in 6 unsuccessfully treated patients (88.2%) (mean: 4.0 ± 1.2 EV; range: 2.4–8.4 EV) (Table 1). The IgA antibody titers were positive in 25 successfully treated patients and in 6 unsuccessfully treated patients (91.2%) (mean: 7.2 ± 6.1 EV; range: 2.3–16.7 EV). Of the patients, 3 (8.8%) were negative for both IgG and IgA antibodies, and 1 (2.9%) was negative for IgG antibodies but positive for IgA antibodies. There

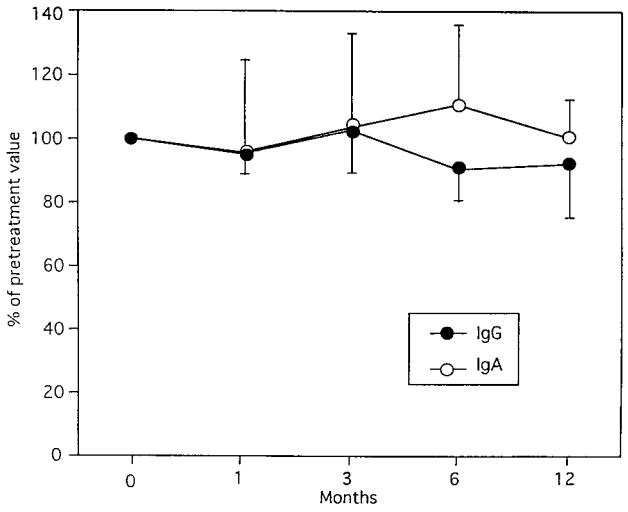

Fig 2. Serum IgG and IgA antibody titers in patients with unsuccessful eradication. Posttreatment titers are expressed as percentages of pretreatment titers.

were no patients with indeterminate values of IgG or IgA antibodies. These seropositive patients (30 patients for the IgG class and 31 patients for the IgA class) were included in this follow-up study. The mean follow-up period for 25 patients with successful eradication was 15 months. In 6 patients with failed eradication, the mean follow-up period was shorter either because of an additional round of eradication therapy or because of dropping out.

Compared with pretreatment titers, the IgG and IgA antibody levels showed a continuous and significant decrease in successfully treated patients throughout the follow-up periods (Fig 1). Mean titer decreases of the IgG and IgA antibodies were 11% and 32% at 1 month ($P < .001$), 25% and 46% at 3 months ($P < .001$), 34% and 52% at 6 months ($P < .05$), 48% and 61% at 12 months ($P < .05$), 43% and 60% at 18 months ($P = .18$), and 57% and 67% at 24 months ($P = .12$). In patients with failed eradication, there was no significant decrease of any antibody titer (Fig 2); 1 patient showed a maximum decrease of 30% of IgG at 6 months and 1 patient showed a 28% decrease of IgA at 3 months. According to an index of successful eradication, sensitivity and specificity of each titer decrease percentage are listed in Table 2. In 1 patient in whom ^{13}C-urea breath test results at 28 months showed reinfection, reappearance of the IgG and IgA antibodies was demonstrated (Table 3).

TABLE 3. Serum IgG and IgA Antibody Titers in a Patient With Reinfection

Follow-up (Months)	^{13}C-UBT* (20 min; ‰)	Antibody Titer (EV)	
		IgG	IgA
0	9.4	2.8	3.5
1	ND	2.4	1.9
3	0.2	1.9	1.8
6	ND	1.6	1.4
12	0.3	0.8	1.2
24	ND	1.5	1.4
28	16.0	2.4	3.0

* ≧4.5‰, positive for urease activity.
ND indicates not done.

Seroreversion rates of IgG and IgA antibodies were 53% and 48% at 12 months' posttreatment and 86% and 81% at 24 months' posttreatment, respectively. Mean periods from the end of eradication therapy to seroreversion of IgG and IgA antibodies were 11.2 ± 7.0 and 11.6 ± 7.8 months, respectively ($P = .76$) (Fig 3). A higher pretreatment titer was significantly correlated with a longer period to seroreversion for IgG antibody ($r = 0.44$; $P < .05$) but not for IgA antibody ($r = 0.15$; $P = .48$).

DISCUSSION

Serum antibodies to *H pylori* antigen, IgG, IgA, and less frequently, to immunoglobulin M classes, are detected in infected individuals.[12] Although such a serum immune response does not act in eradicating the organism, its diagnostic significance (especially of the IgG-specific antibody) is well known.[7,8] Because IgA-specific antibody assayed using a noncommercial method is positive in two thirds of *H pylori*-infected patients, it might be useful only in a small proportion of patients with negative IgG antibody.[12] However, the PP-CAP IgA kit has demonstrated sensitivity and specificity of ~90% in an evaluation of *H pylori* infection.[21] Our study suggests that the diagnostic usefulness of the IgA-specific antibody is not limited to a subset of *H pylori*-infected patients who are IgG antibody negative.

The dynamics of both IgG and IgA antibodies after eradication therapy have been reported in several previous, although somewhat conflicting, studies. Kosunen et al[12] have reported that after eradication therapy IgG antibody titers decrease more regularly than IgA antibody titers, whereas at 6 weeks' posttreatment a titer decrease of the IgA antibody was

TABLE 2. Sensitivity and Specificity of Each Titer Decrease Percentage in Assessing Eradication

	Titer Decrease (%)	IgG			IgA		
		6 Months	12 Months	24 Months	6 Months	12 Months	24 Months
No. of patients		25	22	8	26	23	9
Sensitivity (%)	≥40	40.0	77.8	87.5	71.4*	89.5	100
	≥30	75.0	88.9	100	90.5	94.7	100
	≥20	85.0	94.4	100	90.5	100	100
Specificity (%)	≥40	100	100	ND	100	100	ND
	≥30	80.0	100	ND	100	100	ND
	≥20	80.0	75.0	ND	80.0	100	ND

* $P < .05$ difference compared with values of IgG antibodies.
ND indicates not done.

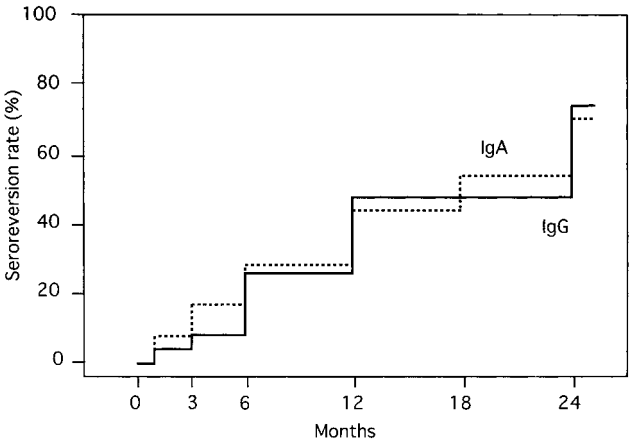

Fig 3. Cumulative seroreversion rates of serum IgG (solid line) and IgA antibodies (broken line) in patients with successful eradication.

greater than that of the IgG antibody. Another study showed that the IgG titer reaches a plateau at 12 Monthsnths' posttreatment.[14] However, in our study, mean decreases of IgG and IgA antibody titers were constant until 24 months with a greater decrease in IgA antibodies. This might be explained by a different immunologic response to *H pylori* between adults and children. Moreover, although an initial decline of IgG and IgA antibody titers also was observed in patients with failed eradication,[12,22] such a change was found in only 2 of our patients with eradication failure. This discrepancy may be attributable to different degrees of transient suppression of *H pylori* colonization after the therapy, but the precise reason is unclear.

A ≥50% decrease of serum IgG antibody titer at 32 weeks from pretreatment values has indicated successful eradication with high sensitivity and specificity.[13] Kosunen et al[12] reported that sensitivity of a 50% decrease at 6 months is 97% for IgG antibodies and 84% for IgA antibodies and that a 60% decrease of each antibody means eradication of *H pylori*. In contrast, as an indicator of eradication, Cutler et al[14] have stressed a decrease in titer of the IgG antibody of 20% at ≥12 months. Our study demonstrates that a ≥30% decrease of IgA antibody at 6 months indicates successful eradication with high sensitivity and specificity. A 30% decrease of the IgG antibody at 12 months' posttreatment was equal to that of the IgA antibody at 6 months. We conclude that serum antibodies should be checked at an endpoint of 6 months or more for assessment of *H pylori* eradication. For this purpose, we believe that the serum IgA antibody is equal to or probably more useful than is the IgG antibody. It is likely that seroreversion of either IgG or IgA antibodies indicates successful eradication.

In a previous, semiquantitative study,[23] approximately half of the patients became IgG antibody negative at 6 months' posttreatment, and nearly all patients became IgG negative at 12 months. Other authors have also reported early seroreversion within 12 months.[11,16] In contrast, 65% of patients remained seropositive at ≥12 months.[14] Veenendaal et al[10] reported that seroreversion rates of IgG and IgA at 12 months were 9% and 45%, respectively. Similarly, the present study has shown that 47% and 52% of patients remain IgG-seropositive and IgA-seropositive at 12 months, respectively, and that seroreversion of IgG and IgA antibodies takes an average of 11 to 12 months. Activity of gastritis rapidly improves after eradication of *H pylori*, but chronic inflammation with mononuclear cell infiltration persists for more than several months.[24] In children with nodular gastritis, histologic inflammatory response does not subside completely in the short-term, despite endoscopic disappearance of antral nodularity.[17] It seems that a majority of successfully treated children gradually become seronegative in parallel with delayed loss of immunologic memory for *H pylori* antigen. This may be linked to a close relationship of a higher pretreatment titer of IgG antibodies with a longer period to seroreversion.

As occurred in our patient, reinfection after successful eradication was accompanied by reelevation of the IgG antibody[15] and of both IgG and IgA antibodies.[10] Although reinfection is rare in older children,[20,25] reelevation or reappearance of the antibodies strongly suggests reinfection. Compared with the ^{13}C-urea breath test, the limitation of the serology test should be noted for assessment of eradication. However, the ^{13}C-urea breath test requires special equipment, it is expensive, and its methodology has not been established for children. The serology test is suitable for children not only because of its noninvasive nature but because of its high reliability.

REFERENCES

1. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet*. 1984;i:1311–1315
2. Blaser MJ. Gastric *Campylobacter*-like organisms, gastritis, and peptic ulcer disease. *Gastroenterology*. 1987;93:371–383
3. NIH Consensus Conference. *Helicobacter pylori* in peptic ulcer disease. *JAMA*. 1994;272:65–69
4. Cutler AF, Havstad S, Ma CK, et al. Accuracy of invasive and noninvasive test to diagnose *Helicobacter pylori* infection. *Gastroenterology*. 1995;109:136–141
5. Thijs JC, Van Zwet AA, Thijs WJ, et al. Diagnostic test for *Helicobacter pylori*: a prospective evaluation of their accuracy with an independent, gold standard. *Gastroenterology*. 1995;108:A241. Abstract
6. Atherton JC, Spiller RC. The urea breath test for *Helicobacter pylori*. *Gut*. 1994;35:723–725
7. Marchildon PA, Ciota LM, Zamaniyan FZ, Peacock JS, Graham DY. Evaluation of three commercial immunoassays compared with the ^{13}C urea breath test for detection of *Helicobacter pylori* infection. *J Clin Microbiol*. 1996;34:1147–1152
8. Feldman RA, Deeks JJ, Evans SJ. Multi-laboratory comparison of eight commercially available *Helicobacter pylori* serology kits. *Eur J Clin Microbiol Infect Dis*. 1995;14:428–433
9. Xia HH, Talley NJ. Natural acquisition and spontaneous elimination of *Helicobacter pylori* infection: clinical implications. *Am J Gastroenterol*. 1997;92:1780–1787
10. Veenendaal RA, Pena AS, Meijer JL, et al. Long term surveillance after treatment of *Helicobacter pylori* infection. *Gut*. 1991;32:1291–1294
11. Cullen DJE, Cullen KJ, Collins BJ, Christiansen KJ, Epis J. Serologic assessment of *Helicobacter pylori* eradication. *Lancet*. 1992;340:1161–1162
12. Kosunen TU, Seppälä K, Sarna S, Sipponen P. Diagnostic value of decreasing IgG, IgA, and IgM antibody titers after eradication of *Helicobacter pylori*. *Lancet*. 1992;339:893–895
13. Hirschl AM, Brandstätter G, Dragosics B, et al. Kinetics of specific IgG antibodies for monitoring the effect of anti-*Helicobacter pylori* chemotherapy. *J Infect Dis*. 1993;168:763–766
14. Cutler AF, Prasad VM. Long-term follow-up of *Helicobacter pylori* serology after successful eradication. *Am J Gastroenterol*. 1996;91:85–88

15. Oderda G, Vaira D, Ainley C, et al. Eighteen month follow up of *Helicobacter pylori* positive children treated with amoxicillin and tinidazole. *Gut*. 1992;33:1328–1330
16. De Giacomo C, Lisato L, Negrini R, Licardi G, Maggiore G. Serum immune response to *Helicobacter pylori* in children: epidemiologic and clinical applications. *J Pediatr*. 1991;119:205–210
17. Kato S, Takeyama J, Ebina K, et al. Omeprazole-based dual and triple regimens for *Helicobacter pylori* eradication in children. *Pediatrics*. 1997; 100(1). URL: http://www.pediatrics.org/cgi/content/full/100/1/e3
18. Rathbone BJ, Wyatt JI, Worsley BW, et al. Systemic and local antibody responses to gastric *Campylobacter pyloridis* in non-ulcer dyspepsia. *Gut*. 1986;27:642–647
19. Kato S, Ritsuno H, Ohnuma K, et al. Safety and efficacy of one-week triple therapy for eradicating *Helicobacter pylori* in children. *Helicobacter*. 1998;3:278–282
20. Kato S, Abukawa D, Furuyama N, Iinuma K. *Helicobacter pylori* reinfection rates in children after eradication therapy. *J Pediatr Gastroenterol Nutr*. 1998;27:543–546
21. Marchildon P, Campbell T, Passaretti N, et al. Evaluation of commercial EIAs for detection IgG and IgA antibodies to *H. pylori*. Xth International Workshop on Gastroduodenal Pathology and *Helicobacter Pylori*; September 1997; Lisbon, Portugal
22. Ashorn M, Ruuska T, Karikoski R, Miettinen A, Mäki M. *Helicobacter pylori* gastritis in dyspeptic children. *Scand J Gastroenterol*. 1994;29: 203–208
23. Wang WM, Chen CY, Jan CM, et al. Long-term follow-up and serologic study after triple therapy of *Helicobacter pylori*-associated duodenal ulcer. *Am J Gastroenterol*. 1994;89:1793–1796
24. Valle J, Seppälä K, Sipponen P, Kosunen T. Disappearance of gastritis after eradication of *Helicobacter pylori*. *Scand J Gastroenterol*. 1991;26: 1057–1065
25. Rowland M, Kumar D, O'Connor P, et al. Reinfection with *Helicobacter pylori* in children. *Gastroenterology*. 1997;112:A273. Abstract

Long-term Follow-up Study of Serum Immunoglobulin G and Immunoglobulin A Antibodies After *Helicobacter pylori* Eradication

Seiichi Kato, Noriko Furuyama, Kyoko Ozawa, Kenji Ohnuma and Kazuie Iinuma

Pediatrics 1999;104;e22
DOI: 10.1542/peds.104.2.e22

PEDIATRICS®
OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

Long-term Follow-up Study of Serum Immunoglobulin G and Immunoglobulin A Antibodies After *Helicobacter pylori* Eradication

Seiichi Kato, Noriko Furuyama, Kyoko Ozawa, Kenji Ohnuma and Kazuie Iinuma

Pediatrics 1999;104;e22
DOI: 10.1542/peds.104.2.e22