Evaluation of Helicobacter pylori Immunoglobulin G (IgG), IgA, and IgM Serologic Testing Compared to Stool Antigen Testing

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Helicobacter pylori causes gastrointestinal disease in both children and adults (10). Noninvasive diagnostic tests include the [13C]urea breath test, serology, and stool antigen testing (HpSA). Numerous studies have evaluated these diagnostic tests but have been limited by a small sample size or restriction to either children or adults (3–9, 11–17, 19, 20). The clinical utility of serologic testing in both children and adults has been debated; moreover, it has not been established whether positive cutoff levels need to be adjusted for age (4, 5, 8, 13, 19). Immunoglobulin A (IgA) and IgG serologic tests are possibly less reliable in children than adults, but this has not been definitively established (13). Some investigators have supported the use of IgM as an indicator of active disease (2), while others have found IgM to have little diagnostic utility (7, 18). Because of the conflicting data, we performed a large-scale study on H. pylori serology to analyze its utility and differences in performance in children and adults.

Paired results of H. pylori serology (IgG, IgA, and/or IgM) and HpSA from October 1998 to January 2009 were analyzed in tests performed within 2 months of each other. HpSA was performed using the Premier Platinum HpSA Plus enzyme immunoassay according to the manufacturer’s instructions (Meridian Bioscience, Inc., Cincinnati, OH). The cutoff optical density at 450 nm was <0.100 for a negative result and ≥0.100 for a positive result.

Serology has been performed with in-house enzyme-linked immunosorbent assay (ELISA) kits used since 1998. The IgG and IgA ELISAs were validated against the Enteric Products Inc. (Stony Brook, NY) ELISA. The IgM ELISA was validated against the MRL (now Focus Diagnostics, Cypress, CA) IgM ELISA. H. pylori antigens (CagA and VacA; Micro Detect, Inc., Tustin, CA) were used to coat microtiter plates at 1.0 μg/ml. Samples were diluted 1:101 for IgG and IgA and 1:51 for IgM and then reacted at room temperature for 30 min. After washing, diluted horseradish peroxidase-conjugated anti-human IgG, IgA, or IgM was reacted for 30 min at room temperature. After washing again, the wells were developed with tetramethylbenzidine for 30 min and the absorbance was measured at 450 nm. The cutoffs (in index values) for IgG and IgA were ≤1.7 for a negative result, 1.8 to 2.2 for an equivocal

| Ig and age group (n) | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Accuracy (%) |
|---------------------|----------------|----------------|---------|---------|--------------|
| IgG                 |                |                |         |         |              |
| All ages (2,423)    | 87.6 (83.0–91.4) | 61.0 (58.8–63.2) | 22.8 (20.4–25.6) | 97.4 (96.4–98.2) | 64.2 (61.4–66.8) |
| ≥17 yr (930)        | 85.6 (75.6–92.6) | 82.6 (79.8–85.2) | 32.0 (25.6–39.0) | 98.4 (97.0–99.2) | 83.0 (80.0–85.8) |
| ≥18 yr (1,493)      | 88.6 (83.0–92.8) | 46.2 (43.4–49.2) | 20.6 (17.8–23.6) | 96.2 (94.4–97.6) | 52.0 (48–56.2) |
| IgA                 |                |                |         |         |              |
| All ages (1,284)    | 63.4 (54.0–72.2) | 67.6 (64.6–70.4) | 17.6 (14.0–21.6) | 94.4 (92.6–96) | 67.2 (63.8–70.4) |
| ≥17 yr (462)        | 29.6 (13.8–50.2) | 95.8 (93.4–97.4) | 30.8 (14.4–51.8) | 95.6 (93.0–97.2) | 91.8 (89.2–94.4) |
| ≥18 yr (822)        | 73.8 (63.4–82.6) | 48.8 (44.8–52.8) | 16.6 (13.2–20.8) | 96.2 (94.4–97.6) | 51.8 (46.4–57.2) |
| IgM                 |                |                |         |         |              |
| All ages (1,015)    | 6.8 (2.6–14.0) | 95.8 (94.2–97.0) | 13.6 (5.2–27.4) | 91.2 (89.2–93.0) | 87.8 (89.2–93.0) |
| ≥17 yr (650)        | 9.0 (2.6–21.6) | 97.0 (95.2–98.2) | 18.2 (5.2–40.2) | 93.6 (91.2–95.4) | 91.0 (88.6–93.2) |
| ≥18 yr (365)        | 4.4 (0.6–15.2) | 93.4 (90.2–96.0) | 9.0 (1.2–29.2) | 87.0 (82.8–90.4) | 82.2 (78–86.2) |

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a Values in parentheses are 95% confidence intervals.

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result, and $\geq 2.3$ for a positive result. For IgM the cutoffs were $\leq 0.8$ for a negative result, 0.9 to 1.1 for an equivocal result, and $\geq 1.2$ for a positive result.

Statistical analyses were performed using SAS software, version 9.1 (SAS Institute Inc., Cary, NC). The study was approved by the Institutional Review Board of the University of Utah (no. 7275).

For all tests performed over the 11-year period, including nonpaired samples, the positivity rate of HpSA (12.1% [10,440/86,284]) was significantly lower ($P < 0.001$) than those for \textit{H. pylori} IgG (35.6% [155,370/413,222]) and IgA (32.7% [60,091/166,997]), and IgM was significantly less often positive (4.3% [5,320/120,135]) than the other three tests ($P < 0.001$) based on the binomial test.

There were 4,722 paired serology and HpSA results for 2,730 women (57.8%) and 1,992 men (42.2%). Eighty-eight percent of these tests were collected within 2 weeks of each other. Using HpSA as the gold standard, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated with 95% confidence intervals for IgG, IgA, and IgM and according to age group: children (≤17 years) and adults (≥18 years) (Table 1). IgG demonstrated the highest sensitivity (87.6%) and lowest specificity (61.0%) and was significantly more specific in children (82.6%) than adults (46.2%). In children, IgA was significantly more specific than adults (95.8% versus 48.8%) but also less sensitive (29.6% versus 73.8%). Overall, IgM demonstrated low sensitivity (6.8%) but high specificity (95.8%) with no statistical difference between children and adults.

A receiver operating characteristics (ROC) curve was generated for each antibody tested (Fig. 1). The ROC area for IgG (0.802) was higher than those for IgA (0.698) and IgM (0.615) ($P < 0.01$, $\chi^2$ test). The ROC area for IgG for children was higher than for adults ($P < 0.001$), as was the ROC area for IgG ($P < 0.01$) (Fig. 2 and 3). Optimal cutoffs were calculated using an iterative method maximizing the product of sensitivity ($1 - \text{specificity}$) (Table 2). No statistically significant differences in ROC curves were noted between male and female patients for IgG, IgA, or IgM serologies.

In this study, IgG correlated better with HpSA than IgA or IgG, IgA, and IgM and according to age group: children (≤17 years) and adults (≥18 years) (Table 1).

| Ig and age group | Optimal cutoff$^a$ | Sensitivity (%) | Specificity (%) |
|------------------|---------------------|-----------------|-----------------|
| IgG              |                     |                 |                 |
| All ages         | 2.94                | 81.2            | 71.8            |
| ≤17 yr           | 2.45                | 80.0            | 85.9            |
| ≥18 yr           | 5.04                | 72.3            | 74.3            |
| IgA              |                     |                 |                 |
| All ages         | 1.62                | 70.0            | 60.4            |
| ≤17 yr           | 0.81                | 70.0            | 78.7            |
| ≥18 yr           | 2.55                | 60.0            | 60.9            |
| IgM              |                     |                 |                 |
| All ages         | 0.27                | 67.0            | 49.2            |
| ≤17 yr           | 0.30                | 34.3            | 65.7            |
| ≥18 yr           | 0.27                | 64.4            | 51.6            |

$^a$ Cutoff values are reported as index values based on analysis of the respective ROC curve with \textit{H. pylori} stool antigen as the gold standard. Cutoffs do not include an equivocal range.
IgM. IgG was also much more specific in children than adults, corroborating the fact that adults are more likely to have been exposed to *H. pylori* in the past (6, 8, 11). While some investigators have observed IgA to be equal to IgG in performance (11), others have found it to have no additional benefit (5, 13). Here, IgA yielded poor overall sensitivity and specificity, although it performed better for samples from children than those from adults. Since serology may not measure active disease, it is not surprising that IgG and IgA were more frequently positive than HpSA, a more accurate indicator of active disease (15). These data are consistent with the low specificity of IgA and IgG serologies.

IgM has been found to have little diagnostic utility for *H. pylori* infections and is elevated only acutely after infection, whereas *H. pylori* infections are generally chronic (8, 18). Here we show that IgM has extremely low sensitivity, confirming its lack of clinical utility in either children or adults.

It has been debated whether cutoffs for serology should be adjusted for children versus adults (5, 18). Titters to IgA and IgG increase with age in response to exposures to *H. pylori* (1, 8). Hence, a lower cutoff may be useful in children to account for this (11). In our analysis, a lower cutoff of 0.8 for IgA in children increased the sensitivity from 29.6% to 70.0% with a lesser decrease in specificity. We recommend that laboratories reevaluate serologic titers based on age to determine if separate cutoffs are warranted. Test characteristics may also vary with different kits and patient population characteristics.

This study was strengthened by the extremely large sample volume but potentially limited by lack of clinical data, which were unavailable. We could not differentiate samples used to establish diagnosis from those drawn for follow-up testing. HpSA was used as the gold standard as it offers excellent sensitivity and specificity when compared to invasive methods, such as gastric biopsy, culture, and the rapid urease test (14, 20). Because CagA and VacA were the antigens used in the serologic testing, it is possible that serology was unable to detect antibodies against certain CagA-negative strains that do not produce vacuolating cytotoxins.

Using HpSA as the gold standard, we found that the performances of IgG and IgA serology tests differ significantly by age. IgG demonstrated the best performance overall. IgM showed little clinical utility, with an acceptably low sensitivity. Our data support the use of different cutoffs for children and adults. Further clinical correlation is needed to establish the optimal cutoffs for these groups.

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