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Evaluation of [^{13}C]Urea Breath Test and *Helicobacter pylori* Stool Antigen Test for Diagnosis of *H. pylori* Infection in Children from a Developing Country

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The [^{13}C]urea breath test (^{13}C-UBT) and *Helicobacter pylori* stool antigen test (HpSA) for the diagnosis of *H. pylori* infection in children were validated. The sensitivity, specificity, and positive and negative predictive values were 93.8, 99.1, 97.8, and 98.0%, respectively, for the ^{13}C-UBT and 96.9, 100, 100, and 98.0%, respectively, for HpSA. Both tests are appropriate for diagnosing *H. pylori* infection in children.

The [^{13}C]urea breath test (^{13}C-UBT) and a new developed immunoassay for the detection of *Helicobacter pylori* antigens in stool, the *H. pylori* stool antigen test (HpSA) (3), are noninvasive tests for *H. pylori* diagnosis.

Although the ^{13}C-UBT has a good sensitivity for the diagnosis of the infection in all ages, low specificity for very young children has been found (6). With respect to the stool test, low sensitivity for this age group has been also reported (2, 10). Thus, we aimed to validate the ^{13}C-UBT and HpSA for the diagnosis of *H. pylori* infection in children.

We studied prospectively 210 consecutive children aged 1 to 18 years who underwent esophagogastroduodenoscopy for investigation of gastric complaints. This project was approved by the Ethics Committee of our institution.

At endoscopy, biopsy specimens were obtained from the antral and oxyntic gastric mucosa for culture, urease test, and histology. Children were considered to be *H. pylori* positive if at least two of the three tests were positive or if the culture alone was positive. They were considered negative if all tests were negative.

The ^{13}C-UBT was performed on fasting children within 1 week after endoscopy. The children received 200 ml of orange juice containing 75 mg of [^{13}C]urea (or 100 ml containing 50 mg for those <30 kg). Breath samples at baseline and after 30 min were analyzed with a nondispersive infrared spectrometer (NDIRIS; Wagner Analysen Technik, Bremen, Germany). The results were considered positive when delta over baseline (DOB) was >4.0‰.

Stool samples, collected on the occasion of the endoscopy, were maintained at −20°C for up 1 year before testing. HpSA (Premier Platinum HpSA; Meridian Diagnostic, Cincinnati, Ohio) was performed according to the manufacturer's recommendation, slightly modified: instead of a stick, a 10-μl disposable loop was used to dilute stool samples, as proposed by Oderda et al. (9), and the plates were washed exhaustively to remove unbound material.

Among the 210 children enrolled, 167 underwent the ^{13}C-UBT but 6 children were excluded (4 had only one positive test and 2 had equivocal results in the ^{13}C-UBT). The remaining 161 children (76 boys; mean age, 8.6 ± 3.8 years; range, 1 to 18 years) were included in the final analysis (48 were *H. pylori* positive and 15 had peptic ulcer). The ^{13}C-UBT was positive for 45 of 48 infected children and for one of the 113 noninfected ones (Table 1). The three children with false-negative ^{13}C-UBT results were a 5-year-old boy (DOB = 0.3), a 9-year-old girl (DOB = 0.5), and a 14-year-old girl (DOB = 0.2). The cultures for all of them were positive. The false-positive result was for an 8-year-old girl (DOB = 8.5). The culture, the histology, and the urease test for this patient were negative. When children were stratified by age into groups of 1 to 6 years (8 *H. pylori* positive and 42 negative) and 7 to 18 years (40 *H. pylori* positive and 71 negative), the sensitivities of the test were 87.5 and 95.0%, respectively, and the specificities were 100 and 98.6%, respectively. No statistical difference between the groups in terms of the sensitivity and specificity of the test was observed.

The high sensitivity and specificity that we observed even in young children may be due to geographic variation among populations or to different protocols. In fact, despite the higher volume of expired air required for the infrared spectrometry we used, it had the same accuracy as mass spectrometry (5, 8) and is less expensive and easier to use. Furthermore, the urea dose of 50 mg (1) employed for children weighing <30 kg in this study minimizes the cost of the test and does not change its accuracy. In addition, the commercial orange juice that we used was accepted readily by the children and did not interfere with the performance of the test.

In this study we observed an inverse correlation between

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TABLE 1. Performance of the HpSA and ^{13}C-UBT for children

Test (*n*)	Sensitivity (95% CI[a])	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
HpSA (107)	96.9 (82.0–99.8)	100 (93.9–100)	100 (86.3–100)	98.7 (91.9–99.9)
^{13}C-UBT (161)	93.8 (81.8–98.4)	99.1 (94.4–99.9)	97.8 (86.2–99.9)	98.0 (91.9–99.3)

[a] CI, confidence interval.

DOB values for the infected patients and age (Pearson's correlation; $r = -0.47$, $P = 0.001$) but not between age and DOB values for noninfected children. An inverse correlation between age and DOB values for both infected and noninfected children has been observed by different authors, which may explain the low specificity of the test seen in young children (6, 7). The differences among the studies in the group of negative children may be due to differences among populations (2), and these differences may explain the high specificity that we observed in all age groups studied.

HpSA was performed for 107 of 161 patients who underwent the ^{13}C-UBT. HpSA was positive for 31 of 32 *H. pylori*-positive children (Table 1). When children were stratified by age into groups of 1 to 6 years (7 *H. pylori* positive and 27 negative) and 7 to 18 years (25 *H. pylori* positive and 48 negative), the sensitivities were 100 and 96%, respectively, and the specificity for both groups was 100%. Besides the high specificity we observed, the test also showed high sensitivity for detecting *H. pylori* infection in children of all ages. It is possible that children from developing countries have higher bacterial loads and larger amounts of *H. pylori* antigens in the stools, both of which would improve the sensitivity of the tests; if this is the case, it reinforces the necessity of validating the methods currently used for the diagnosis of *H. pylori* infection in different geographical areas. It has to be emphasized that in this study the samples were stored at −20°C immediately after they were obtained, a fact that may contribute to better conservation of *H. pylori* antigens in the stool samples and to the high sensitivity observed.

There was no correlation between the optical density from the HpSA (OD) for the infected children and the age ($P = 0.9$) or between the ODs for the infected children with and without duodenal ulcer ($P = 1.0$). Conversely, in concordance with the literature (4, 7), a significant correlation between OD and DOB values (Pearson's correlation; $r = 0.495$; $P = 0.04$) for *H. pylori*-positive children was observed.

The HpSA results were in concordance with the ^{13}C-UBT for 31 of 32 *H. pylori*-infected children and for all 75 noninfected children (kappa coefficient = 0.978).

In conclusion, we demonstrated that the noninvasive tests ^{13}C-UBT and HpSA are highly concordant and specific for the diagnosis of *H. pylori* infection in children of all ages. However, a large number of young children need to be evaluated since we studied few infected children in this age group.

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