COMPARISON OF NON-INVASIVE TESTS TO DETECT *HELICOBACTER PYLORI* INFECTION IN CHILDREN AND ADOLESCENTS: RESULTS OF A MULTICENTER EUROPEAN STUDY

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Objective **To compare the current non-invasive tests for *Helicobacter pylori* infection in children and adolescents.**

Study design **This multicenter, multinational study investigated the sensitivity, specificity, and positive and negative predictive values of four non-invasive tests: urea breath test (UBT), stool antigen test, and antibody detection in serum and urine, in comparison with biopsy-based tests.**

Results **Of 503 patients included pre-treatment, 473 fulfilled the definition of *H pylori* status and among those 316 had results available for the four non-invasive tests (including 133 *H pylori*-positive patients). The specificity was excellent for all tests. The UBT had the best sensitivity in all age groups, followed by serology, stool test, and antibody detection in urine. A trend for better sensitivity with an increase in age was observed except for the stool test. The receiver operating characteristics (ROC) curves showed that sensitivity of serology, stool test, and urinelisa could be improved by changing the cutoff value. An inadequate storage of the specimens may explain the poor results of the stool test.**

Conclusion **The UBT appears to be an excellent test for diagnosis of *H pylori* infection for children and adolescents.** ***(J Pediatr 2005;146:198-203)***

Helicobacter pylori is acquired early in life, and it persists for decades and maybe even lifelong. The chronic gastritis that it induces may not be symptomatic but is considered to be the background of severe diseases, ie, peptic ulcer disease and gastric malignancies that typically occur in adulthood. Although duodenal ulcer disease is rarely found in children, it does occur and, as in adults, can be the consequence of an *H pylori* infection. Moreover, *H pylori* infection has been incriminated in other syndromes, ie, recurrent abdominal pain and iron deficiency anemia, but remains to be confirmed, and for this purpose it is mandatory to compare the value of non-invasive tests.

The *H pylori* prevalence in childhood reflects the prevalence that will be found in adulthood in a given age cohort. There is a great contrast between developed countries, where only very few children are infected, and developing countries, where most children reach adulthood being *H pylori* positive.[1]

The need for an accurate non-invasive test in children to study the transmission of the disease and to monitor the success of eradication therapy by groups in Europe and in North America.[2,3] There are now four types of tests available on the market, two based on detection of specific antibodies, in serum and urine, one based on the detection of *H pylori* antigen in stool, and the urea breath test (UBT), which detects the strong urease activity of this bacterium. Although several studies have already been performed on children, the four non-invasive tests have never been compared in the same study with a "gold standard" including all biopsy-based tests. In addition, the number of patients included in previous studies has not been sufficient enough to allow a meaningful analysis of age subgroups, especially for children <6 years of age in which the information is the most important to establish.[4]

See editorial, p 164.

METHODS

This is an open, prospective multicenter study investigating the diagnostic properties of non-invasive tests in comparison with a gold standard for the diagnosis of *H pylori* infection in children (2-5 and 6-11 years of age) and adolescents (12-17 years of age).

UBT	Urea breath test	ROC	Receiver operating characteristics
HpSA	*H pylori* stool antigen test		

From the European Paediatric Task Force on *Helicobacter pylori*.

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Patients between 2 and 17 years of age were included if an upper digestive tract endoscopy and *H pylori* testing was required. An informed consent was obtained from the legal guardian and when possible from the patient. Patients were excluded in the case of previous *H pylori* eradication therapy, consumption of antibiotics, antisecretory drugs, bismuth salts, or sucralfate in the previous 2 weeks, or if they manifested coagulopathy or any other disorder leading to a contraindication for endoscopy and/or biopsies.

The size of the study population was calculated to be 600 children and 200 adolescents in order to have at least 30 *H pylori* positive patients per age group (children 2-11 years of age, and adolescents 12-17 years of age) and to demonstrate with a probability of 90% that sensitivity and specificity were >85% using one-sided confidence intervals.[5] It was decided that the study could be stopped when more than 30 *H pylori* positive patients would be included in each age group.

The following diagnostic tests were performed within 1 week:

Endoscopic examination with at least five biopsies sampled. Four biopsies were sampled from the antrum (one each for local culture, central culture, histology, and rapid urease test) and one from the corpus for histology. Biopsies for histology were processed and interpreted blindly according to the Sydney System[6] in the local laboratory of each participating center.

In addition to culture performed locally, one antral biopsy per patient was stored frozen at −80°C and transported in dry ice twice during the study to a central laboratory in Bordeaux where they were processed blindly according to a protocol previously described.[7]

The rapid urease test (RUT) used was PyloriTek (BARD, Billerica, Mass).[8] Reading was performed within 1 hour.

Definition of *H. pylori* status. A positive *H pylori* status was defined as a positive culture (either local or central, or both) or in case of negative culture, positive results for both histology and rapid urease test. A negative *H pylori* status was confirmed when all invasive tests performed gave concordant negative results. Cases with discrepant results, ie, histology positive and rapid urease test negative or vice versa, were excluded.

^{13}C-urea breath test. The Helicobacter Test INFAI (INFAI GmbH, Cologne, Germany) was used.[9] The test was performed on fasting children (>4 hours after their last meal and at least 2 hours after the endoscopic examination). The test meal used was 200 mL of orange or apple juice: 150 mL of juice was given; thereafter, ^{13}C-labeled urea dissolved in 20 mL of juice was administered and another 30 mL of juice was used to rinse the mouth of the tracer. A total of 100 mL of juice was given to children 2 to 4 years of age. The ^{13}C-urea dosage was 75 mg for adolescents and 45 mg for children. The analysis was performed blindly on expired air samples collected before and 30 minutes after urea ingestion, by mass spectrometry centrally (INFAI Laboratory, Cologne, Germany). A δ over baseline value of >4 per mil was considered to be the threshold for positivity.

Table I. Characteristics of the 316 children and adolescents studied with known *Helicobacter pylori* status and the four tests performed

	N	% *H pylori* +
Total	316	42.1
Gender		
Male	144	38.9
Female	171	44.4
Not reported	1	
Age (y)		
2-5	48	27.1
6-11	150	44.7
12-17	118	44.9
Mean age 9.9 ± 3.7		
Ethnic status		
Caucasian	293	39.2
African	4	100
Asian	1	0
Other	13	84.6
Not reported	5	
Reason for endoscopy*		
Pain	261	47.9
Symptoms of malabsorption	35	20.0
Vomiting	65	26.1
Failure to thrive	19	42.1
Other	64	32.8
Endoscopy finding		
Duodenal ulcer	10	100
Gastric ulcer	2	100
Duodenal erosion	14	64.2
Gastritis erosion	12	41.7
Duodenal nodules	24	79.1
Gastritis nodules	131	83.2
Normal endoscopy	123	16.2

*Some children had several reasons to be endoscoped.

Stool antigen test. The kit Premier *H pylori* Stool Antigen test (HpSA) (Meridian, Milan, Italy) was used.[10] Stool samples were obtained from patients, frozen at −20°C, transported frozen under the manufacturer's responsibility separately from the other specimens, and processed blindly in a central laboratory (Y. Glucpzynski, Yvoir, Belgium).

Serology. The kit Pyloritest EIA-G III (Orion Diagnostics, Espoo, Finland) was used. Serum samples were kept frozen at −20°C, transported in dry ice in a central laboratory in Bordeaux, and tested blindly.

Antibody detection in urine. The kit Urinelisa (Otsuka Diagnostic, Frankfurt, Germany) was used.[11] Urine samples were collected in a conservation medium and sent by regular mail to a central laboratory in Bordeaux, where they were processed blindly.

Table II. Performances of the diagnostic tests for the 316 patients with gold standard and four tests performed (UBT, HpSA, Urinelisa and Pyloriset EIA-G)

	Age group (y)	Helicobacter test INFAI	HpSA	Urinelisa	Pyloriset EIA-G	**Rapirun
Sensitivity	Global	96.2 [91.9-98.6]	72.9 [64.9-80.0] ***80.3 [73.0-86.5]	63.2 [54.7-71.0] ***72.2 [64.1-79.3]	88.7 [82.5-93.3] ***90.2 [84.2-94.4]	30.2 [22.5-38.9]
Specificity	Global	97.3 [94.0-99.0]	97.3 [94.0-99.0] ***93.4 [89.1-96.4]	97.3 [94.0-99.0] ***93.4 [89.1-96.4]	93.4 [89.1-96.4] ***93.9 [89.1-96.4]	98.7 [95.7-99.8]
Accuracy	Global	96.8 [94.4-98.4]	87.0 [83.0-90.4]	82.9 [78.4-86.8]	91.5 [88.0-94.2]	68.7 [63.0-74.0]
PPV	Global	96.2 [91.9-98.6]	95.1 [89.5-98.2]	94.4 [88.0-97.9]	90.8 [84.8-94.9]	94.7 [83.6-99.1]
NPV	Global	97.3 [94.0-99.0]	83.2 [77.7-87.7]	78.4 [72.7-83.4]	91.9 [87.3-95.2]	64.5 [58.2-70.4]

NPV, Negative Predictive Value; *PPV*, Positive Predictive Value.
**Rapirun was performed only on 272 children including 119 *H pylori* positive. Age groups were the following: 2-5 y (n = 41 including 11 *H pylori* positive), 6-11 y (n = 130 including 59 *H pylori* positive) and 12-17 y (n = 101 including 49 *H pylori* positive).
***Sensitivity and specificity values obtained after defining the optimal cutoff based on ROC curves.

A test called "near patient" (Rapirun, Otsuka Diagnostic, Tokyo, Japan) also was performed locally on a subgroup of patients.

All kits were used according to the manufacturer's instructions. The proposed cutoff was used in the evaluation. A calculation of the receiver operating characteristics (ROC) curves also was performed for the non-invasive tests. ROC curves allow for the determination of the threshold, giving the best combination of sensitivity and specificity for a given test.

This study followed the requirements for good clinical practice. It was approved by the ethical committee of each of the participating institutions.

Statistical Analysis

Sensitivities, specificities, predictive values, and diagnostic accuracy were determined separately for each diagnostic test: Helicobacter Test INFAI, Urinelisa, Pyloriset-EIA-G III, HpSA, and Rapirun. Diagnostic accuracy was calculated as the percentage of the patients who were scored correctly (true positive and true negative) among all patients tested.

A ROC curve analysis was used to determine the best cutoff value for each diagnostic test. Results were expressed with 95% CI. A P value of <.05 indicated statistical significance. Statistical analysis was performed using STATA 7.0 statistical software (Stata Corporation, College Station, Tex).

RESULTS

Of 503 patients recruited in the study, 473 fulfilled the definition of a positive or negative *H pylori* status and 316 had further results available for the four non-invasive tests. There were 191 *H pylori* positive cases among the 473 with defined *H pylori* status; culture was positive for 167 (88%), both locally and centrally in 74% of them, and locally or centrally in 13%, and an additional 24 cases were both urease and histology positive. Chronic gastritis was present in all. The characteristics of the population of 316 children and adolescents for which results were available for the four non-invasive tests are presented in Table I.

The sensitivity, specificity, accuracy, and positive and negative predictive values of the different tests, on this population of 316 children and adolescents, are presented in Table II. The results were not different from those of the 473 regarding all of the characteristics studied. The ROC curves indicated that the cutoff values proposed by the manufacturers were not optimal for HpSA and Urinelisa. Indeed, the sensitivity of HpSA could be increased to 80.3%, and the sensitivity of Urinelisa to 72.2%, both with a minor loss in specificity by decreasing the cutoff values (Figure).

When the sensitivity of the four tests was compared according to the three age groups, a trend for a better sensitivity in adolescents compared with children was observed for all tests except HpSA. It was more marked for Urinelisa, but it did not reach statistical significance (P = .2).

In order to know the predictive values of the tests in centers with the lowest prevalence, we carried out a subgroup analysis in which 76 cases were included. The positive and negative predictive values were the following: UBT: 76.4% and 98.3%, Pyloriset-EIA-G III 76.4% and 98.3%, HpSA 83.3% and 93.7%, Urinelisa 83.3% and 93.7%, Rapirun 84.7% and 75%, respectively. The accuracy of the tests also was calculated using as reference the combination of the histology and RUT instead of culture. The accuracy was then 88.7% for UBT, 83.7% for Pyloriset-EIA-G III, 78% for HpSA, 73.7% for Urinelisa, and 59.5% for Rapirun.

DISCUSSION

Because of the current rarity of symptomatic *H pylori* infection in this period of life, previous studies did not include large enough numbers of young patients to break down the results by age. In this study we could differentiate adolescents from children. However, we could not include enough children between 2 and 5 years of age to obtain reliable results.

The rate of *H pylori* infection observed in this study is high and cannot be interpreted as the prevalence rate in children in Europe. It can be explained mainly by a strong recruitment of immigrant children in centers of Western

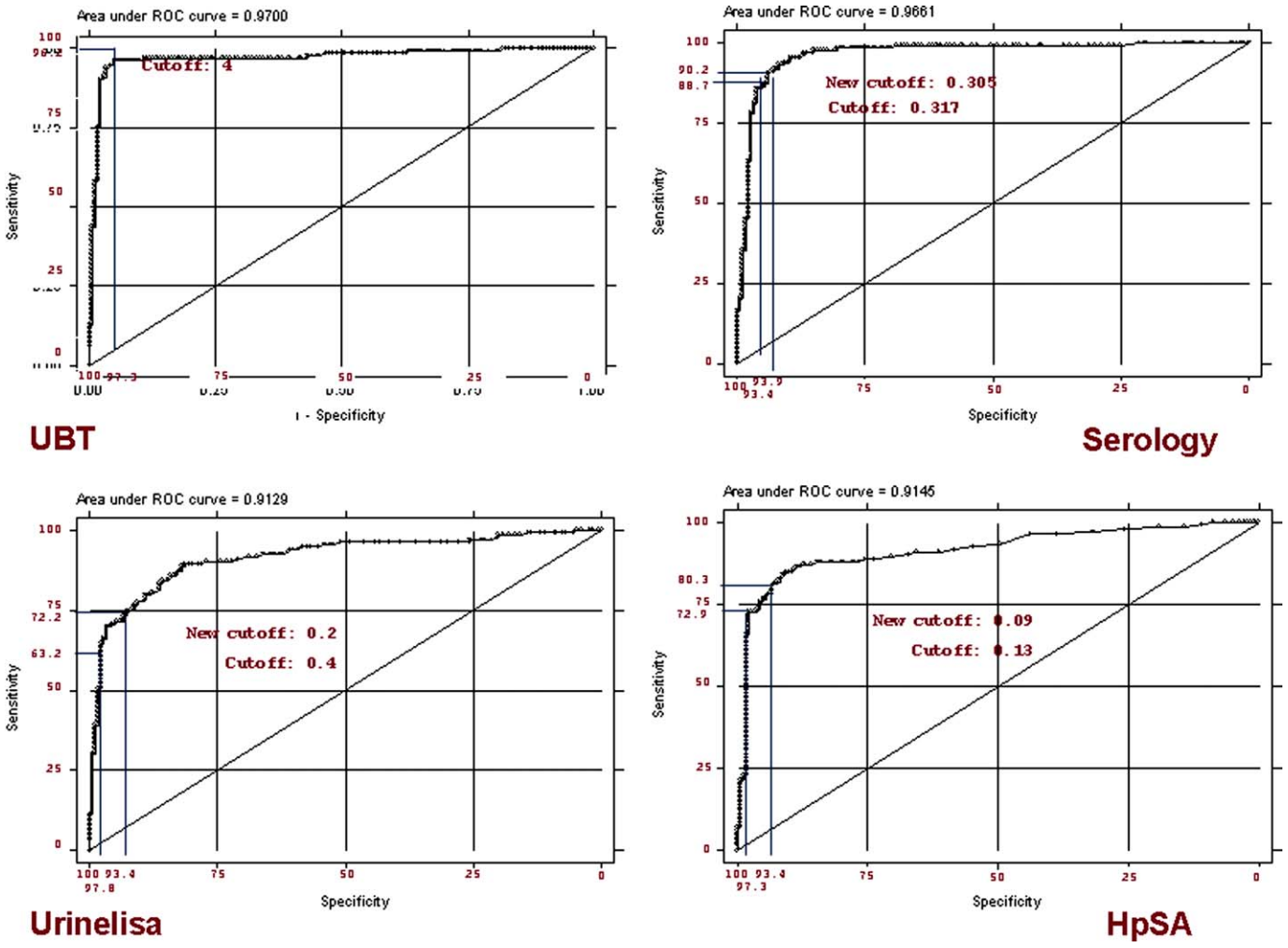

Figure. ROC curve of four non-invasive tests in comparison with reference biopsy-based tests for diagnosis of *H pylori* infection. Sensitivity and specificity are calculated for each value, which is then plotted. The curve allows for determination of the best cutoff.

Europe. Therefore, it must be acknowledged that the predictive values of the test performances may vary according to local *H pylori* prevalence. For this reason we calculated the positive and negative predictive values for the centers with a low prevalence of the infection, but the small number of cases limited the power of the results.

A relatively low rate of ulcers was noted (8.6%), but they were all *H pylori* positive. The rate was however higher than in a series of 622 upper endoscopy reports in the United States in which only 11 case patients had an ulcer and only 3 of them an *H pylori* positive ulcer.[12]

A strong point of this study is the reliability of the *H pylori* status used as a gold standard. Culture was performed meticulously and turned out to be highly successful despite the fact that the centers were spread all over Europe. Among the 191 gold standard positive cases, 167 were positive by culture. In case of a negative culture usually explainable by a transport problem or lack of experience for certain centers, the other criterion used was to have both urease test and histology positive. The urease test chosen has been recognized as the most sensitive and practical test,[13] and in this study, it also showed an excellent performance when compared with culture.

The evaluation concerned four different non-invasive tests based on three different principles, given that Serology and Urinelisa used the same principal, ie antibody detection. The results confirm the value of UBT, which exhibits an excellent sensitivity in all age groups, as previously demonstrated in several studies.[14-16] We did not notice a lower specificity in young children in our small sample in contrast with previous data,[17,18,19] and all of the DOB values of *H pylori* negative cases were far from the cutoff (<1.7 δ per mil), but this result must be interpreted with caution given the low number of such cases. The use of 45 mg of ^{13}C-urea in children is therefore justified, as well as is the protocol consisting of a test meal of orange or apple juice after fasting with rinsing the mouth after tracer ingestion. The cutoff of 4 per mil is also the best alternative. These results confirm previous data presented by Bazzoli et al,[20] who obtained the same results with 50 mg as with 100 mg of urea when performing the UBT in children.

A surprising finding of this study was the low sensitivity of the HpSA. This test has confirmed its value in the past both

in adults[21] and in children,[22-27] especially for pretreatment diagnosis. The stability of the antigens to be detected has been previously mentioned by the manufacturer and confirmed in a study in which bacteria were experimentally spiked in stools.[28] Therefore, in this study the recommendation was made to freeze the specimens at −20°C only. They were then transported frozen to the central laboratory performing this test. It must be acknowledged that, for customs reasons, some samples arrived thawed. In addition, asking parents to bring in stool specimens introduces a lack of full control on the time between defecation and storage in the ward. Therefore, the possibility of inadequate storage may explain the poor results. The falsely negative results, however, were randomly distributed among the centers and occurred throughout the study period, which does not suggest problem limited to certain shipments. If *H pylori* antigens present in stools are not stable, the requirement for maintenance before testing must be reinforced in everyday practice in order to ensure proper results. An alternative hypothesis would be that the quality of the reagent, a polyclonal antibody, was different in this study compared with previous ones. Inter-test variability has been previously described.[29] However, by lowering the cutoff, it was possible to increase the sensitivity of HpSA to 80.3%. Recently, a novel *H pylori* antigen stool test using monoclonal antibodies has shown very promising results (sensitivity 98%, specificity 99%), when applied to a similar patient population without variation according to age, and the problem of lot variation.[30]

Serology ranked second in sensitivity. The kit was initially chosen on the basis of a previous evaluation in which it had the best accuracy.[31] It was confirmed to be excellent in our laboratory for adult patients,[32] and it now proves to be true for the children in this study. It also may be that the third generation of this test has a higher accuracy compared with the previous ones.[33] Serologic tests have a bad reputation, which could be linked to two reasons: the variability of accuracy between kits, and the fact that they suffer from a comparison to a "low quality" gold standard because culture is rarely performed and histology may not be accurate. The outcome is then an apparent lack of specificity, whereas most false positives are probably true positives. The other antibody test, used on urine samples, did not perform well on children. The amount of antibodies in urine reflects the amount present in serum, but at a lower level. It is therefore logical that this test would be less sensitive than serology. Indeed, we found 44 cases positive in serum and negative in urine, versus only 3 cases negative in serum and positive in urine. Interestingly, the sensitivity increased significantly with age, reflecting a higher antibody response in adolescents than in children. Decreasing the cutoff allowed a notable increase in the sensitivity. The current kit is based on antigens from Japanese strains, which are known to have a special genetic pattern. It may well be that their antigen spectrum is different from that of most European strains isolated in this study, causing the low sensitivity that was not noticed in Japan.[34] The urine "near patient" test, which could be the ideal non-invasive test if its performance were satisfactory, exhibited a very low sensitivity as occurs with "near patient" blood tests compared with laboratory enzyme linked immunosorbent assays.[35]

In conclusion, all of the tests showed a trend for improved sensitivity with age except for the stool test. Of the methods evaluated in this study, UBT is the non-invasive test of choice, but more data are needed for children <5 years of age. Serology using Pyloriset EIA-G gave satisfactory results.

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APPENDIX

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