Evaluation of a Commercial Immunoblot, Helicoblot 2.1, for Diagnosis of Helicobacter pylori Infection

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The best method to diagnose Helicobacter pylori infection in different clinical situations is controversial. The aim of the study was to assess the performance of a commercial immunoblot, Helicoblot 2.1. The study comprised 215 patients, who were grouped according to the presence of H. pylori infection (assessed by two gastroscopies including histology with a median interval of 7.1 years, enzyme immunoassay [EIA]-based serology, and history of previous H. pylori infections and eradication therapies) into four categories: no H. pylori infection ever, previous infection, ongoing infection, and EIA seropositivity as the only marker of a possible previous infection. The sensitivity of Helicoblot 2.1 to show an ongoing or previous H. pylori infection was 100% and 92%, respectively. Helicoblot 2.1 was negative in only 80% of individuals with no evidence of present or previous infection but in 96% of patients 50 years of age or younger. The current infection marker of the immunoblot was positive in 49% of patients with successful H. pylori eradication therapy. After successful eradication therapy, Helicoblot 2.1 sustained positive results in 87% of patients, and CagA positivity was detected in 87% of patients with follow-up samples for more than 10 years after therapy. Helicoblot 2.1 is a sensitive and, among patients of ages 50 years or younger, a specific test in the primary diagnosis of H. pylori infection. However, it does not discriminate between past and current infections. It can be used in epidemiological studies assessing the role of H. pylori in different late sequelae.

Helicobacter pylori infection is clearly associated with peptic ulcer disease, gastric cancer, and gastric mucosa-associated lymphoid tissue lymphoma (17). None of the available diagnostic methods for H. pylori infection are ideal (23). The invasive methods, such as rapid urease test, histology, and culture, have high specificity but low sensitivity in the presence of atrophic gastritis and during proton pump inhibitor (PPI) therapy (22). The same is true for the noninvasive urea breath and stool antigen tests. Serology is recommended to be used in atrophic gastritis and during PPI therapy, but a single serum sample does not usually differentiate the past and ongoing infections. Even 30% of patients had elevated antibodies of the immunoglobulin G (IgG) class still after 5 years of successful eradication therapy (43).

The true impact of H. pylori on gastric cancer incidence is not really known (21, 31). Patients with advanced atrophic gastritis have the highest cancer risk (39), but the best method for diagnosing a past H. pylori infection in gastric atrophy is debatable. CagA antibodies are thought to best reflect the past infections and were shown to sustain for longer than other H. pylori antibodies in a follow-up of 32 months (40). However, the specificity of CagA antibodies has been challenged (38). Furthermore, the role of H. pylori in a severe autoimmune type of gastric atrophy also remains unresolved (1).

Several immunoblotting methods for the diagnosis of H. pylori infection are available, but the Helicoblot test is probably the most studied (11). The previous studies of the performance of the Helicoblot test in the diagnosis of an ongoing H. pylori infection have shown variable results. In children, the sensitivities of Helicoblot 2.0 (32, 34) and Helicoblot 2.1 (26, 28) varied between 95.5 and 100% and 80 and 98.6%, respectively, and the specificities between 85.7 and 88% and 87.1 and 100%, respectively. In adults, Helicoblot 2.1 showed sensitivities of 93.4 to 99% and specificities of 88 to 98% (12, 18, 25, 30, 44). After eradication therapy, the seroreversion rates have been low, less than 10% (14), and the current infection marker (CIM) has been shown to be unreliable (18). The accuracy of Helicoblot 2.1 in determining the CagA or VacA status compared to that of the genotyping has been variable, and in many studies, the use of the test for this purpose has not been recommended (8, 15, 30).

The aim of this study was to evaluate the performance of the commercial immunoblot Helicoblot 2.1 in a series of patients, both in the primary diagnosis of H. pylori infection and with follow-up data available for about 7 years after eradication therapy. We also evaluated the role of CagA antibodies in the diagnosis of a past H. pylori infection. Helicoblot 2.1 was a sensitive and specific test in the primary diagnosis of H. pylori infection, especially in younger patients. It had a low discrimination value in the assessment of the success of H. pylori eradication therapy. Because H. pylori antibodies detected by Helicoblot 2.1, especially those to CagA, remained for years after successful eradication therapy, immunoblotting seemed to be the most sensitive method to detect a past H. pylori infection.

MATERIALS AND METHODS

Patients. During the 2-year period from 1 January 2004 to 31 December 2005, a total of 345 consecutive patients with at least one previous upper gastrointes-
tinal endoscopy a median of 7.1 years earlier underwent gastroscopy, and 235 of
the patients also gave serum samples. Of the 235 patients, 94 had no signs of a
tinal endoscopy a median of 7.1 years earlier underwent gastroscopy, and 235 of
the patients had had unsuccessful eradication therapies earlier. In an additional 20
additional 84

TABLE 1. Characteristics of the patients grouped according to \textit{H. pylori} status

| Characteristic | No known infection (n = 94) | Previous infection (n = 84) | Ongoing infection (n = 27) | Elevated \textit{H. pylori} antibodies only (n = 10) |
|---------------|----------------------------|-----------------------------|---------------------------|-----------------------------------------------|
| Median age (yr) | 61.3                       | 65.6                        | 64.9                      | 69.3                                          |
| Age range (yr) | 24–85                      | 28–87                       | 42–82                     | 26–79                                         |
| Median time between gastroscopies (yr) | 7.4 (78)               | 6.7 (74)                     | 9.9 (18)                  | 6.1 (9)                                       |
| (no. of patients with data available) |                      |                             |                          |                                               |
| Female/male (%) | 68 (72)/26 (28)            | 66 (79)/18 (21)             | 19 (70)/8 (30)            | 5 (50)/5 (50)                                 |
| No. of patients with following main endoscopy findings (%) |                     |                             |                          |                                               |
| Abdominal pain | 31 (33)                    | 37 (44)                     | 10 (37)                   | 4 (40)                                        |
| Reflux | 28 (30)                     | 17 (20)                     | 4 (15)                    | 4 (40)                                        |
| Dyspepsia | 11 (12)                     | 8 (10)                      | 6 (22)                    | 0                                             |
| Anemia or diarrhea | 7 (7)                      | 2 (2)                       | 1 (4)                     | 1 (10)                                        |
| No. of patients with following macroscopic findings (%) |                     |                             |                          |                                               |
| Erosive esophagitis/Barrett | 10 (11)                    | 12 (14)/1 (1)               | 3 (11)/0                  | 1 (10)/0                                     |
| Erosive gastritis/ulcer | 8 (9)/1 (1)                | 9 (11)/1 (1)                | 2 (7)/3 (11)              | 2 (20)/0                                      |
| Duodenal erosion | 2 (2)                      | 1 (1)                       | 0                         | 1 (10)                                        |
| No. of patients with following histologic findings (%) |                     |                             |                          |                                               |
| Chronic gastritis | 9 (10)                     | 29 (35)                     | 27 (100)                  | 1 (10)                                        |
| Atrophic corpus gastritis | 1 (1)                      | 3 (4)                       | 5 (19)                    | 0                                             |
| No. of patients with PPI use on daily basis (%) | 17 (18)                    | 13 (15)                     | 1 (4)                     | 0                                             |
| No. of patients with no PPI use (%) | 4 (4)                      | 5 (6)                       | 2 (7)                     | 0                                             |
| No. of patients with NSAID use (%) |                     |                             |                          |                                               |
| Less than once a mo | 15 (16)                    | 11 (13)                     | 4 (15)                    | 1 (10)                                        |
| More than once a wk | 17 (18)                    | 15 (18)                     | 3 (11)                    | 1 (10)                                        |

- NSAID, nonsteroidal anti-inflammatory drug.

**RESULTS**

The overall performance of Helicoblot 2.1 in patients with no known \textit{H. pylori} infection, those with an ongoing \textit{H. pylori} infection, those with a past \textit{H. pylori} infection, and those with

**Histology.** During the gastroscopy of the present study, two biopsies were taken from both antrum and corpus for histology. Biopsies were stained with hematoxylin and eosin, Alcian blue (pH 2.5)-periodic acid-Schiff, and modified Giemsa stain and assessed according to the updated Sydney System by one experienced pathologist (P.S.) unaware of the identity of the samples (3).

**Serum tests.** The serum specimens of the 215 patients were stored at –20°C until examined. Both IgG and IgA antibodies to \textit{H. pylori} were determined by an in-house enzyme immunoassay (EIA) method as described earlier (27). The sensitivities and specificities compared to the histology results have been 99% and 93% for the IgG antibodies and 64% and 98% for IgA antibodies, respectively (27).

The immunoblot test was performed, and the results were assessed by one laboratory assistant according to the manufacturer’s instructions. The interpretation criteria for an \textit{H. pylori} seropositive sample were as follows: (i) fulfilling the criteria for CagA positivity (namely, the presence of a 116-kDa CagA band in combination with CIM, with the 30-kDa UreA band and the 19.5-kDa band, or with the 89-kDa VacA band, the 37-kDa band, or the 35-kDa band); (ii) the presence of any of the bands at 89 kDa, 37 kDa, or 35 kDa; and (iii) the presence of both the bands at 30 kDa and 19.5 kDa. The intensity of the bands was graded with a naked eye from 1 to 3, grade 1 being defined as a very faint barely visible band.

All patients gave their written informed consent, and the study was approved by the local ethics committee.
TABLE 2. Numbers of patients, grouped according to *H. pylori* status, with positive bands in Helicoblot 2.1 results

| Positive reactions | No known infection (n = 94) | Previous infection (n = 84) | Ongoing infection (n = 27) | Only elevated *H. pylori* antibodies (n = 10) |
|--------------------|-----------------------------|-----------------------------|---------------------------|---------------------------------------------|
| *H. pylori* positive | 19 (20)                     | 77 (92)                     | 27 (100)                  | 7 (70)                                      |
| *H. pylori* and CIM positive | 5 (5.3)                   | 41 (49)                     | 22 (81)                   | 5 (50)                                      |
| CagA 116-kDa band positive | 24 (26)                   | 75 (89)                     | 26 (96)                   | 8 (80)                                      |
| With faint bands excluded | 19 (20)                   | 62 (74)                     | 25 (93)                   | 6 (60)                                      |
| CagA positivityb | 16 (17)                     | 73 (87)                     | 26 (96)                   | 7 (70)                                      |
| VacA 89-kDa band positive | 14 (15)                   | 62 (74)                     | 26 (96)                   | 7 (70)                                      |
| UreA 30-kDa band positive | 15 (16)                   | 60 (71)                     | 27 (100)                  | 4 (40)                                      |
| UreB 61-kDa band positive | 91 (97)                   | 84 (100)                    | 27 (100)                  | 10 (100)                                    |
| HSP 56-kDa band positive | 90 (96)                   | 84 (100)                    | 27 (100)                  | 10 (100)                                    |

a The intensity of the band was assessed by naked eye as faint if the band was barely visible.
b CagA positivity was determined according to the manufacturer’s criteria (also, the faint bands are included).

DISCUSSION

Helicoblot 2.1 showed high sensitivity in detecting *H. pylori* antibodies in all but one presently *H. pylori*-infected subjects and very high specificity (96%) in young patients (≤50 years old). Thus, Helicoblot 2.1 could be used as a confirmatory test in controversial clinical situations. However, antibodies to the CIM band poorly differentiated the patients with past and ongoing infections, as half of the patients with successful eradication therapy showed a positive CIM band. The antibodies detected by the immunoblot persisted, in most cases, for years even after successful eradication therapy, which enables the detection of past infection in epidemiological studies.

The well-characterized patients with follow-up data available for years with no known *H. pylori* infection detected in either previous or present gastroscopy or laboratory tests and no known eradication therapy seemed to show a high prevalence of previous infections, according to the immunoblot analysis results. However, this was the case only in the older patients. Helicoblot 2.1 was actually very specific (96%) in the group of patients 50 years of age or younger. The prevalence of *H. pylori* infection is rapidly declining in Finland (33) and in other developed countries in young-age cohorts. As the positive predictive value of the test is strongly dependent on the prevalence of the disease, the proportion of false-positive test results relative to the true-positive results may be a problem with many of the noninvasive *H. pylori* tests (42). Helicoblot 2.1 could be a confirmatory test for the discrepant results in the younger age group.

The interpretation of the false-positive Helicoblot 2.1 results in the age group older than 50 years is difficult. It is tempting to speculate that the false-positive test results could actually be because of remnants after a spontaneous disappearance of the infection. Another possibility is that, for some reason, Helicoblot 2.1 has low specificity in older age groups, an unresolved problem also contemplated previously (9). Studies comparing the accuracy of the Helicoblot test with that of PCR methods in determining the CagA status have given discrepant results (15, 25, 30, 47), and the reliability of the immunoblot test to verify the CagA status has actually been challenged (37). In a study assessing the gastric histology, most patients with CagA-
positive serum samples were suggested to have real previous infections (49). In our study of 215 patients (excluding the patients with severe corpus atrophy and duodenal ulcer history, because of their ambiguous H. pylori status), 104 patients had no known H. pylori infection or eradication therapy earlier and no H. pylori in histology in the present study. Ten of these 104 patients had positive H. pylori EIA serology (IgG and/or IgA antibodies elevated), but 23 (22%) were CagA positive according to the Helicoblot 2.1 criteria (the CagA band was positive in 32 more patients [31%]). These results are in accordance with studies suggesting a much higher spontaneous disappearance rate of the H. pylori infection (19) than has previously been anticipated to happen (4% in 21 years) (16). If these particular patients are at an increased risk for H. pylori infection, associated long-term sequelae remains to be studied.

In our study, CagA antibodies indeed sustained for longer after successful eradication than did antibodies to other antigens, in accordance with some previous studies (18, 40). The prevalence of CagA positivity, however, slightly declined with time, as the presence of antibodies to the other antigens, needed to fulfill the manufacturer’s criteria for CagA positivity, declined more rapidly. Antibodies solely to the 116-kDa CagA antigen (not fulfilling the criteria for CagA seropositivity according to the manufacturer) were detected in 11 (5%) patients of the whole 215-patient study population, and 2 of them were known to have been previously infected with H. pylori. In studies assessing the impact of H. pylori infection on gastric cancer risk (6, 5, 10, 29, 35, 41, 48, 50) or on the development of atrophic gastritis (1, 4, 7, 24, 36), the different definitions of the criteria for CagA positivity as a sign of a previous contact with H. pylori may give different risk estimations. In our study, among patients for whom it had been more than 5 years since successful H. pylori eradication, the positive EIA serology found only 18 (34%) of the 53 patients with verified past H. pylori infection, but the immunoblot detected 45 (85%) subjects, if CagA positivity according to the manufacturer’s criteria were used, and even more, 47 (89%) subjects, if antibodies solely to CagA antigen had been considered; the specificities were 83% and 74%, respectively. This high sensitivity is in accordance with a previous study (30), which, however, showed very low specificity when the interpretation criteria were changed. In our study, Helicoblot 2.1 had good sensitivity compared to that of EIA serology in detecting a past infection also when the manufacturer’s criteria were followed.

It has been suggested that faint positive reactions to CagA antigen could be false positive (38). However, in our study, the exclusion of patients with antibodies giving only faint reactions to CagA did not significantly increase the overall performance of the test. The specificity of the test only increased from 74% to 80% in patients with no known H. pylori infection, but in patients with previous H. pylori infection, the exclusion of faint bands dropped the sensitivity from 89% to 74%. The unreliability of the CIM band for differentiating ongoing and past H. pylori infections was in accordance with an earlier study showing positive CIM in 80% of patients 3 years after successful eradication therapy (18). Furthermore, in our study, this could be confirmed in patients with substantially longer follow-up periods.

The heat shock protein (HSP) 58-kDa band (13, 46) has been associated with different gastroduodenal diseases, in particular, gastric atrophy (2, 20). It has also been associated with intensity of chronic inflammation in the antrum (45). In our study, antibodies to the 58-kDa antigen were present in 96% of the patients with no known previous H. pylori infection.

In conclusion, Helicoblot 2.1 seems to be a sensitive and specific test in the primary diagnosis of H. pylori infection, especially in younger patients. It has, however, a low discrimination value in the assessment of the success of H. pylori eradication therapy. Because H. pylori antibodies detected by Helicoblot 2.1, especially those to CagA, remain for years after successful eradication therapy, immunoblotting seems to be the most sensitive method to detect a past H. pylori infection. Future studies are needed to clarify the impact of Helicoblot 2.1 as a screening method to define the importance of a past H. pylori infection on the increased risk of gastric cancer or atrophic gastritis.

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