The reliability of ELISA in predicting *H. pylori* infection in dyspeptic populations under age 45

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Summary

Background: Dyspepsia is a common problem worldwide. Many dyspeptic patients have no alarm symptoms and are diagnosed by family doctors. Non-invasive testing of *H. pylori* is recommended in patients up to the age of 55. Testing of asymptomatic high risk groups is now being considered in many countries.

The aim of the study was to compare the accuracy of serology tests (ELISA, HelicoTest) against the benchmark UBT (urea breath test) in the detection of *H. pylori* infection in young populations.

Material/Methods: The study was conducted on 238 patients under age 45 (159 with undiagnosed dyspepsia and 79 controls). The gold standard of diagnosis of *H. pylori* infection was based on a positive UBT. UBT, serological ELISA and HelicoTest were performed on each of the patients.

Results: By UBT 50.9% and by ELISA 49.7% of dyspeptic patients were infected with *H. pylori*, while in the control group 38% and 39.2% were infected, respectively. HelicoTest showed the highest positive results in both groups (55.3% study group, 44.3% control). The average level of IgG antibodies from ELISA testing among patients with a positive UBT *H. pylori* result was 3.16 U/ml. Patients with negative UBT results showed an average IgG level of 0.53 U/ml.

Conclusions: There was a correlation of increasing antibody level on ELISA and probability of testing positive on UBT. The majority of patients had moderate dyspeptic symptoms. The ELISA is an easy, cheap and effective method for diagnosing *H. pylori* infection in the primary care setting.

key words: *Helicobacter pylori* (*H. pylori*) • ELISA test • urea breath test (UBT) • dyspepsia • primary care

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BACKGROUND

The prevalence of dyspeptic complaints in the community is high-up to 25–40%. Some symptom fluctuation occurs in the short term, but troublesome gastrointestinal complaints persist in up to 90% of subjects over a 1–6 month period [1]. Dyspepsia is defined as chronic or recurrent pain or discomfort centered in the upper abdomen. In many countries, the appropriate patient age for use of non-invasive diagnostic strategy is under discussion [2–4]. In clinical practice, *Helicobacter pylori* (*H. pylori*) infection is very common and causes a variety of symptoms, therefore its diagnosis should be based on the best evidence. *H. pylori* infection is associated with peptic ulcer disease, and infection appears to be a protective factor against food allergy [5]. Since its discovery, *H. pylori* has been implicated in a variety of extradigestive diseases – some published data support certain infectious agents as potential risk factors for schizophrenia, but its potential role in the pathogenesis of psychiatric disorders has thus far been largely neglected [6].

An additional non-invasive diagnostic test for *H. pylori* infection has a high yield, over and above gastroscopy, in young patients [7]. There are 2 options for treating patients under age 45 with uninvestigated dyspepsia: either test-and-treat for *H. pylori* or perform a trial of acid suppression [8]. The UBT and stool antigen tests are the recommended diagnostic methods due to their having the highest positive and negative predictive values; however, these tests are not always accessible to primary care physicians. The Maastricht III-2005 Consensus report recommended serology as an alternative option for countries with a high prevalence of *H. pylori* infection [9]. *H. pylori* infection rates vary greatly around the world, ranging from over 80% in Asia to 30% in Germany. A study from the Czech Republic found a 17% prevalence of long-lasting (over 12 months) dyspeptic symptoms in the general population and 38% prevalence of *H. pylori* infection in a group 25–44 years of age [10]. There are many countries with similar sociodemographic determinants. There is a requirement to conduct studies within general practice to be aware of serology tests in GP communities. Serology testing for *H. pylori* is widely used, inexpensive and non-invasive. There are 2 frequently used serology tests: the laboratory ELISA test and rapid blood test. The serology tests have the lowest cost per correct diagnosis at low (30%), intermediate (60%) and high prevalence (90%) of *H. pylori* infection, but diagnostic accuracy is lower than other non-invasive tests. This is in contrast to the UBT, which is more costly, delivers results less quickly and needs special preparation by the patient, such as fasting and cessation of antibiotics for 4 weeks, proton pump inhibitors (PPI) for 2 weeks, and H_2 blockers for 48 hours [11]. The physician involved in the care of undiagnosed dyspeptic patients needs to be aware of the value of these serology tests in young patients.

The aim of this study was to compare the accuracy of serology tests (ELISA, HelicoTest) against the benchmark UBT as a gold standard test in the detection of *H. pylori* infection in patients under the age of 45 who presented to a primary care physician with dyspeptic symptoms for at least 6 months. The additional aims of the study were to determine the frequency of *H. pylori* infection amongst dyspeptic patients under the age of 45 and to estimate the type and severity of symptoms among patients.

MATERIAL AND METHODS

The study was conducted in a primary care setting with patients 20–45 years of age. Each patient underwent the following procedures: history and physical examination, C13 UBT test, serological laboratory ELISA-DPC test, HelicoTest, a rapid test on a drop of blood taken from a finger designed for quick detection of IgG antibodies, and an epidemiological questionnaire using the Glasgow Dyspepsia Severity Score (GDSS). The GDSS includes global ratings of symptoms for dyspepsia including frequency of symptoms, the effect of dyspepsia on normal activities, frequency of medical consultations, use of prescribed medications, and tests for dyspepsia [12]. The GDSS is used as a multidimensional outcome measure assessing dyspepsia syndromes in many clinical trials. Patients presented to primary care physicians in Cracow with undiagnosed symptoms that had been present for at least 6 months. The most frequent symptoms of dyspeptic patients were epigastric pain, upper abdominal discomfort before and after the meal, heartburn and dysmotility-type symptoms (bloating, nausea, belching). The research was conducted from 2004 to 2006. There were 2 study groups – a group with dyspeptic symptoms and a control group selected by computer randomization.

The study group consisted of 159 patients under age 45 who had reported dyspeptic symptoms to GP doctors. The control group consisted of 79 patients under age 45 selected randomly from a total of 7,000 patients listed with 3 GP practices. Both groups were from similar demographic populations in Cracow. The study was approved by the Ethics Committee of Jagiellonian University. All participants gave written informed consent.

Exclusion criteria were: weight loss, anaemia, haematemesis, melena, abdominal tumor, use of NSAIDs, use of antibiotics or ranitidine bismuth citrate 4 weeks prior to the investigation, use of PPI 2 weeks and H_2 blockers 48 hours before investigation. Patients with alarm symptoms and a suspicion of peptic ulcer disease were referred for gastroscopy. The gold standard of diagnosis of *H. pylori* infection was based on a positive UBT test.

Statistical analysis

Statistical analysis was carried out using the STATISTICA and SAS programmes. The agreement of the tests was assessed by 3 criteria: the percentage of incompatible results, the value of the kappa coefficient and McNemara's test for related dichotomous variables. $P<0.05$ was considered statistically significant. Distinguishing a positive or a negative *H. pylori* result was objective in UBT, and ELISA was considered positive at the level of 1.00 U/ml. Patients characteristics are shown in Table 1. In the study group there were more females than males, which is typical for most studies involving patient commitment.

RESULTS

UBT showed that in the study of 159 dyspeptic patients, 50.9% were infected with *H. pylori*, whereas in the control group of 79 patients selected from GP`s lists 38.0% were infected. ELISA tests were positive in 49.7% of patients in the dyspeptic group and 39.2% in the control group. HelicoTest

Table 1. The two patient groups' characteristics.

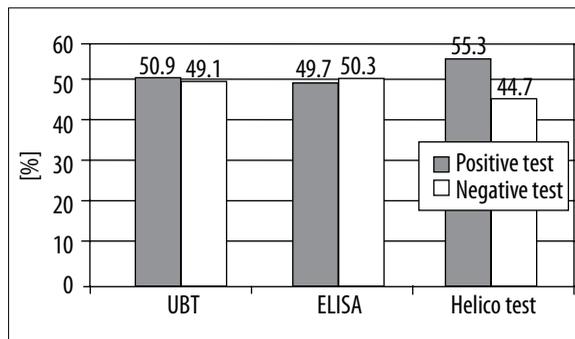|	Age		Sex	
	Mean	**Min.–Max.**	**Female**	**Male**
Study group 159 patients	34.79	20–45	73.6%	26.4%
Control group 79 patients	34.19	20–45	49.4%	50.6%

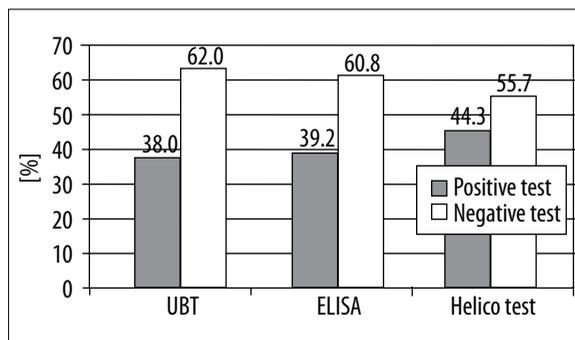**Figure 1.** Prevalence of *H. pylori* infection in the study group p>0.05.

Figure 2. Prevalence of *H. pylori* infection in the control group p>0.05.

Figure 3. Prevalence of *H. pylori* infection in the study group under and over 30 years.
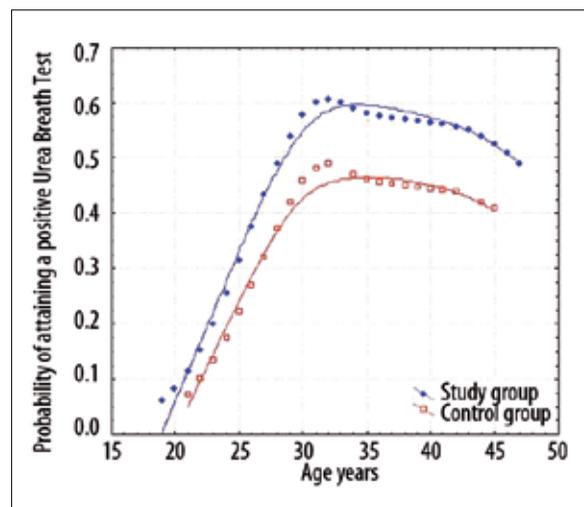
Figure 4. Age-dependent prevalence of *H. pylori* infection in both groups of patients.

Table 2. The probability of *H. pylori* infection in UBT, ELISA and HelicoTest in the study group, control group and all patients.

Test	Study group n=159	Control group n=79	All n=238
UBT	0.51	0.38	0.42
ELISA	0.50	0.39	0.43
HelicoTest	0.55	0.44	0.48

was positive in 55.3% and 44.3% of patients, respectively. Figures 1 and 2 show the prevalence of *H. pylori* infection in the study group and the control group.

H. pylori infection was less common among patients under age 30, with 27.3% vs. 60.0% infected in the over-30 group. Figure 3 shows the prevalence of *H. pylori* infection in populations under and over 30 years (p<0.05).

Prevalence of *H. pylori* infection increased with age in both groups of patients. Figure 4 shows the age-dependent increased prevalence of positive *H. pylori* infection based on UBT testing.

For ELISA tests, assuming a positive result as an antibody level of 1.12 (U/ml) using the GLZ model (Generalized Linear Model) and 1.07 (U/ml) using the GAM model (Generalized Additive Model), the probability of receiving a positive UBT test was 0.5. The probability of receiving a positive UBT rose steeply with increasing antibody level on ELISA. From a 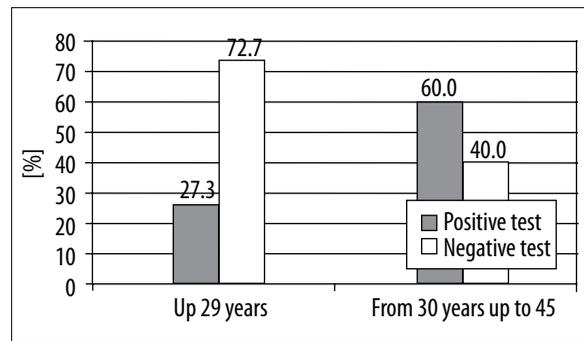probability of 0.5 at the threshold for a positive ELISA test, the probability rises to 0.96 at an antibody level of 2.00 U/ml. The average level of IgG antibodies from ELISA testing among patients with a positive UBT *H. pylori* result was 3.16 U/ml. Patients with negative UBT results showed an average IgG level of 0.53 U/ml. The probability of *H. pylori* infection in each test is shown in Table 2.

The most common patient complaints were moderate dyspeptic symptoms: epigastric pain, upper abdominal discomfort before and after a meal, dysmotility-type symptoms which occurred not more than 2–3 times a week. A minority of patients had acute symptoms occurring every day and experienced mild symptoms 2–3 times a month.

Table 3. Types of symptoms in the study population.

Study group	Type of dyspeptic symptoms [%]			
	Acute	Moderate	Mild	Not specific
159	18.2	59.8	17	5.0

The majority of the young adult population tested had moderate dyspeptic symptoms. Analysis of the data showed little difference in detection of *H. pylori* among patients with acute, moderate or mild dyspeptic symptoms, although *H. pylori* infection was more common in patients with a higher Glasgow Dyspepsia Severity Score. Types od symptoms in the study population are shown inTable 3.

The average level of IgG antibodies in UBT positive patients with acute dyspepsia was 4.18 U/ml, 2.83 U/ml with moderate and 3.26 U/ml with mild. The average level of IgG antibodies in women was 1.76 U/ml and in men 2.02 U/ml in the dyspeptic group, and 1.18 U/ml and 2.05 U/ml, respectively, in the control group. Anti-ulcer medications (anti-acid drugs, H2-blockers or PPI) were taken by 39% of patients. The drugs did not affect the reliability of the ELISA test. The average level of IgG antibodies among patients taking drugs for dyspepsia was 1.9 U/ml vs. 1.7 U/ml in those not taking drugs for dyspepsia (χ^2=3.95, p=0.266).

Figure 5 shows the incompatibility of ELISA and HelicoTest in study and control groups. The difference between the UBT and ELISA tests was from 3.8% to 8.8%, respectively, and the difference between the UBT and HelicoTest was from 16.5% and 13.2%, respectively.

DISCUSSION

The data in this study confirm that prevalence of *H. pylori* infection in young dyspeptic patients is not extremely high, but increased with age. In our study the prevalence of *H. pylori* infection confirmed by UBT was 38% to 50.9%, depending on the group. The prevalence of *H. pylori* in patients with dyspepsia is similar to that reported in the literature, from 28% to 61% [13–15]. A 2000–2003 Polish study by Celiński et al. showed a higher prevalence of *H. pylori,* ranging from 62.8% in large cities, up to 73% in district towns in 20–29 year olds, and 75–88% in individuals up to age 49 [16]. In our study the prevalence was lower, but the exclusion criteria limited persons with early *H. pylori* diagnostic procedures. We also did not include patients with alarm symptoms because of their need for invasive diagnostic procedures. Few studies have been published examining uninvestigated dyspepsia in patients under age 45 in primary care settings in Eastern and Central Europe.

In many countries patients under ages 45, 50 or 55, with dyspepsia and without alarm symptoms, who contacted their primary physician, should be tested for *H. pylori* infection or be given medication to control the symptoms [17,18]. The optimal age threshold for endoscopy is unclear, but 55 years rather than 45 years now seems to be a reasonable cut-off age since stomach cancer is rare in young patients [19].
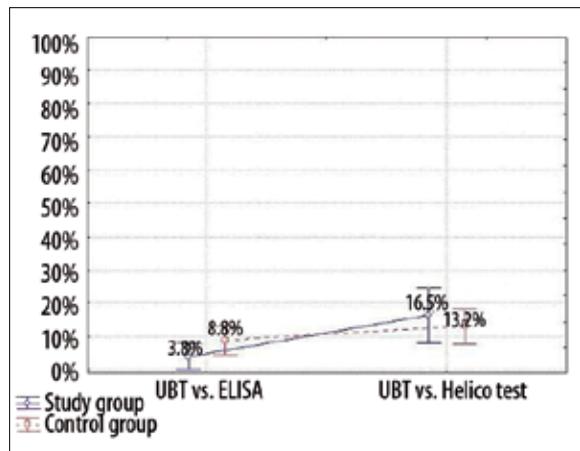

Figure 5. Range of incompatibility between UBT, ELISA, and HelicoTest.

Non-invasive *H. pylori* testing is even more important now than in the past, and diagnosis of the infection is still important in primary care. Past studies have shown that in a population with a high prevalence of *H. pylori* infection, the "test and treat" strategy is the best option. In countries where the prevalence is below 20%, acid suppression is the treatment of choice. Sonnenberg [11] noted that if the ulcer disease prevalence rate exceeded 10% in *H. pylori* positive patients, the non-invasive strategy based on serological testing become more cost-effective. A strategy using empirical PPI can be recommended if the alternative is an endoscopy strategy for managing dyspeptic patients in general practice [20]. The *H. pylori* prevalence in many countries is still high and results of research with respect to diagnostic problems are crucial. In our study population the prevalence in both groups was above 20%.

The results show that there is a small difference between UBT and ELISA in young patients with uninvestigated dyspepsia. Our examinations show that in both groups there was a similar range of prevalence diagnosed by UBT and ELISA; therefore, these results provide strong evidence supporting use of ELISA as a primary diagnostic test in the primary care setting.

This is in contrast to the HelicoTest, which was 55.3% positive in the study group and 44.3% positive in the control group. The range of incompatibility between UBT and HelicoTest was from 16.5% and 13.2%, respectively, indicating they were higher than UBT and ELISA.

In some cases tested with the HelicoTest it was difficult for physicians to differentiate positive from negative results after 10 minutes, and more time was needed. Recent developments in *H. pylori* diagnosis include a commercial immunochromatographic test and immunoblot test covering the current infection marker CIM. Presence of anti-CIM Ig G antibody is highly predictive for active *H. pylori* infection [21]. UBT is now becoming recognized as an excellent test because of its accuracy and robustness. Specimens can be transported without special precautions and the result is independent of human interpretation. The cost of UBT in our study was 28$ and the cost of ELISA was from 5–7$. The prices of UBT and ELISA are not stable, and current costs of UBT and ELISA are 43$ and 9–10$, respectively.

Studies from 2006 on the stool antigen test compared monoclonal vs. polyclonal antibody-based enzyme immunoassay stool tests, and the results were not generally good. Pretreatment specificity was low, 70.7% in the study by Domingues et al. [22], and post-treatment specificity was 73% in a study by Quesada [23]. In many countries access to stool antigen testing in primary care setting is limited.

The new classification of functional dyspepsia by Suzuki is based on a patient-centered approach, with the highest index of symptoms, and the early diagnosis can be based on patient symptoms alone [24,25]. Dyspepsia can present in 25% of the general population as epigastric pain, discomfort, nausea, vomiting, bloating, belching and burning retrosternal pain. A 2008 Kalixanda general population study [1] showed that more than half of subjects reported some type of gastrointestinal complaint. A strategy of *H. pylori* screening and eradication in high-risk populations to reduce gastric cancer incidence has been initiated [26,27], demonstrating that the problem is increasing over time. Interestingly, the distribution of dyspeptic complaints was similar among patients with and without organic findings during upper endoscopy. Invasive diagnostic procedures, therefore, are not always the best solution. In the study we used active (UBT) and passive (serology) non-invasive tests. Serology tests provide evidence of exposure to *H. pylori* antibodies which can stay in the blood for a long time and do not indicate whether the infection is currently active. We studied how these 2 tests compare in young dyspeptic patients without previous eradication treatment. The results showed good correlation in prevalence of passive and active infection in both groups, and correlation with increasing level of antibodies.

Primary care physicians need easy, cost-effective methods to confirm the course of symptoms, *H. pylori* still being the most common. Non-invasive and reliable methods are preferred if no alarm symptoms are present. A major limitation of this study was that patients with symptoms of dyspepsia were included in the control group, which may have increased the percentage of infections.

CONCLUSIONS

ELISA serological testing is a reliable alternative to UBT in the detection of *H. pylori* infection in a population under 45 years of age in the primary care setting. This result was confirmed in both study groups. The HelicoTest was a less reliable method of assessment. ELISA and UBT showed a similar rate of positive results of infection in both groups. The difference in rate of *H. pylori* infection between control and study groups shows a need for treatment of infection in the primary care setting. There is a correlation between ELISA level of IgG antibodies and the probability of a reliable positive UBT test result.

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