Invasive tests for *Helicobacter pylori* in children

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One of the primary indications for upper gastrointestinal (GI) endoscopy in children is the presence of persistent and severe upper abdominal symptoms. Upper GI endoscopies are performed to allow the physician to confirm or rule out upper GI pathology. Additionally, upper GI endoscopies with mucosal biopsies are the gold standard for the diagnosis of *Helicobacter pylori* infection and its complications in children. The gastric biopsies can be used for the rapid urease test, histological examination and bacterial culture to determine antibiotic sensitivity. DNA extracted in these biopsies can also be subjected to genotyping using molecular methods to determine the presence of *H pylori* infection, antibiotic resistance mutations and *H pylori* virulence factors.

Key Words: *Children; Endoscopy;* H pylori*; Histology; Invasive; Molecular diagnostics*

Tests effractifs pour *Helicobacter pylori* chez les enfants

L'une des premières indications de l'endoscopie des voies digestives hautes chez les enfants est la présence de symptômes persistants et sévères. L'endoscopie des voies digestives hautes permet au médecin de confirmer ou d'écarter un diagnostic concernant cette portion de l'appareil digestif. De plus, l'endoscopie, avec biopsie de la muqueuse, constitue la norme diagnostique pour le dépistage de l'infection à *Helicobacter pylori* et de ses complications chez les enfants. Les biopsies gastriques peuvent être utilisées pour le test rapide à l'uréase, et l'examen histologique et la culture bactérienne permettent de déterminer la sensibilité aux antibiotiques. L'ADN extrait des spécimens de biopsies peut également être soumis à un génotypage grâce aux méthodes moléculaires pour confirmer la présence d'une infection à *H pylori*, une mutation entraînant sa résistance aux antibiotiques ou ses facteurs de virulence.

Currently, upper gastrointestinal (GI) endoscopy with mucosal biopsies remains the gold standard for the diagnosis of *Helicobacter pylori* infection in children. The added advantage of endoscopy includes the detection of upper gastrointestinal pathologies including complications of *H pylori* infection such as nodular gastritis, peptic ulcer disease and gastric cancer, and mucosa-associated lymphoid tissue lymphoma. The biopsies obtained can be used for bacterial culture, determining antibiotic sensitivity and bacterial genotyping. The need for anesthesia in children, the high cost and small risks of perforation and aspiration pneumonia are some of the disadvantages of endoscopy.

One of the primary indications for upper GI endoscopy in children is the presence of persistent and severe upper abdominal symptoms, to detect upper GI tract pathologies and not simply the presence of *H pylori*. It is not clinically possible to differentiate between complications in *H pylori*-infected children and other upper GI pathologies or functional dyspepsia. In a retrospective cohort study (1), 52 of 2550 children who had undergone upper GI endoscopy were found to have peptic ulcers (1). Apart from vomiting (found more frequently in those without pathology), no statistical difference was noted in symptomatology between those with and without peptic ulcers. In the subgroup with peptic ulcer disease, symptoms did not differ between *H pylori*-infected and -noninfected individuals (1,2). Nevertheless, children with a family history of peptic ulcer disease are more likely to have *H pylori* infection (3).

The gastritis secondary to *H pylori* infection is not always evident endoscopically, but can be seen in histological examination of gastric biopsies. The most common endoscopic finding in children with *H pylori* infection is nodular gastritis, often seen more in the antrum than the body of the stomach (4). Nodular gastritis can be identified when the gastric mucosa has an irregular 'cobblestone' appearance, highlighted with blood from a bleeding biopsy site. There is a significant association between nodular gastritis and *H pylori* infection. Nodular gastritis was found in 45% of *H pylori*-infected and 1.5% of -noninfected children (4), and its prevalence increased with age (5). The authors of these studies (4,5) concluded that nodular hyperplasia or gastritis is highly specific (98.5%) and, therefore, has a high positive predictive value for *H pylori* infection, but low sensitivity (44%). The absence of nodular hyperplasia does not preclude the presence of infection. In addition, there is a positive correlation among nodular hyperplasia, severity of gastritis and bacteria density (4).

TESTS FOR DETECTING *H PYLORI*

Biopsy site

At least two biopsies from one or more regions of the stomach (body, antrum and transitional zone, ie, cardia and incisura) are required for diagnosis (6). In patients who have not received treatment, an antral biopsy has a much higher yield than biopsies obtained from the body in *H pylori* detection (94% versus 73%, respectively). The region that has the highest detection rate is the midantrum region of the lesser curvature (6). Often biopsies from the transitional zone and body are also required to improve the yield in patients who have been treated with acid suppression therapy or antibiotics (7,8). For

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those with complications of *H pylori* infection, such as peptic ulcer disease, it is recommended to have biopsies from multiple regions of the stomach.

Histological staining of biopsy section

Histological detection of *H pylori* remains an affordable and reliable method in comparison with other assays (9). In the majority of cases, *H pylori* can be seen with hematoxylin and eosin staining; however, this method has a lower sensitivity and most laboratories use alternative staining methods for improved detection. Polyclonal anti-*H pylori* antibody staining is regarded as a reliable assay; however, this method is expensive and time consuming (10). In comparison, the Giemsa stain, although less reliable, is widely available, affordable and can be readily prepared. The optimal staining method is often best determined by the expertise available locally.

Rapid urease tests

Numerous urease tests are commercially available. Urease is a metalloenzyme produced in abundance by *H pylori* (11). Urease converts urea to ammonia, thereby increasing the pH in the bacterium's microenvironment. Urease tests are highly specific and sensitive for indirectly detecting *H pylori* infection in adults (11). In children, however, its sensitivity is much lower and false-negative results are produced. The lower sensitivity could be secondary to reduced urease activity in *H pylori* strains isolated from children and lower bacterial load (12). The accuracy of urease tests, like histological examination, is also dependent on the number of biopsies taken, sites of biopsy and the use of antibiotics and proton pump inhibitors (13).

***H pylori* culture**

H pylori is a fastidious organism and is difficult to culture from gastric biopsy. Bacterial culturing is also time consuming and expensive. In one study (14), culture tests had a low sensitivity, with positive results in only 70% of *H pylori*-infected children. However, bacterial culture tests allow for antibiotic sensitivity to be determined, which is particularly useful in those who have failed previous eradication therapy. Various antibiotic susceptibility tests are also available, including the epsilometer (E)-test, disc test and agar dilution methods. The Clinical and Laboratory Standards Institute (formerly known as the National Committee for Clinical Laboratory Standards) recommends agar dilution as the method of choice for antibiotic sensitivity testing in *H pylori* infection (15). A positive culture test also allows for the opportunity to genotype clinical isolates for specific virulence factors (a method employed in the research setting).

Molecular diagnostics using gastric tissue

Over the past few years, advances have been made using rapid molecular diagnostics to detect *H pylori* infection, antibiotic sensitivity, the presence of specific virulence factors and the patient's genotype (as reviewed by Simala-Grant and Taylor [16] and Ruzsovics et al [17]). These molecular diagnostic tests may allow for more cost-effective and individualized treatment, resulting in improved eradication rates, and reduction in antibiotic-resistant strains.

Molecular methods for detecting *H pylori* in gastric biopsies

The polymerase chain reaction (PCR) is highly sensitive in detecting microbes in human tissue. Using paired primers directed at a region of the *urease* A gene or *glm*M (*ure*C gene) of *H pylori* (10,18), it was found that although nested PCR performed on gastric biopsy specimens was sensitive, it was not as specific as histopathology and the rapid urease test for detecting *H pylori* infection.

Recently, three studies (19-21) have used real-time PCR to detect and quantify *H pylori* infection directly from gastric biopsy specimens. He et al (19), using this technique with a LightCycler apparatus (Roche Diagnostics, USA) and a frozen section, amplified a fragment of the *ure*C gene, but a low specificity was achieved (a high number of specimens were positive by real-time PCR but negative by culture and histology). Lascols et al (20), however, demonstrated with the same apparatus that targeting the 23S ribosomal (r)RNA gene in frozen section by real-time PCR gave a sensitivity of 97% and a specificity of 94.6% (infection was defined as a positive culture, histology or positive PCR, if confirmed by a positive concomitant serology or urea breath test). In addtion, Kobayashi et al (21) used the *Tag*Man apparatus and a paraffin section to target the 16S rRNA gene and had both the highest sensitivity and specificity (100%) compared with culture tests, histology, the urea breath test and rapid urease test. The detection of *H pylori* on gastric specimens by real-time PCR is very promising; however, more clinical studies are needed to determine the optimal target gene and confirm its reliability.

Molecular methods to detect *H pylori* with antibiotic resistance genotypes

PCR, when performed on cultured clinical isolates or directly on gastric biopsies obtained from infected individuals, has been used to determine the antibiotic sensitivity of *H pylori* strains, particularly with respect to clarithromycin. The predominant cause of clarithromycin resistance is a point mutations in the peptidyl transferase of the 23S rRNA gene (22). Using LightCycler real-time PCR to detect these point mutations, Chisholm et al (22) demonstrated a strong correlation between wild type and clarithromycin sensitivity in 47 of 48 strains of *H pylori*. Four strains had clarithromycin resistance mutations, but only two demonstrated resistance when assessed by disk diffusion. There were four PCR-negative specimens, and three were sensitive to clarithromycin; the reliability of this test was unclear. However, in a recent study, Lascols et al (20) showed a very high concordance rate of 98.3% (58 of 59) comparing clarithromycin susceptibility testing with E-test and LightCycler real-time PCR. The prevalence of clarithromycin resistance was 18.5% in that study. Thus, the use of real-time PCR in the detection of *H pylori* with clarithromycin resistance genotypes is likely to be a valuable tool.

Fluorescent in situ hybridization using labelled oligonucleotides can be used to detect *H pylori* by probing for *H pylori*-specific 16S rRNA and point mutations in the 23S rRNA of clarithromycin-resistant strains (23). This technique can be used in both frozen as well as formalin-fixed and paraffin-embedded gastric biopsy sections, and is reliable in detecting clarithromycin-resistant *H pylori* (24-26).

Mutations in the *rdx*A gene encoding for a nitroreductase (which converts metronidazole from a harmless prodrug to a bactericidal agent) may result in *H pylori* resistance to metronidazole (27). In other reductase genes, such as *fdx*B (encoding for ferrodoxin-like protein) and *frx*A (encoding for flavin

nitroreductase), inactivating mutations enhance metronidazole resistance (28). It is likely that there are other reductases that are involved in *H pylori* resistance to metronidazole (29-31). These studies (29-31) suggest that there are multiple *H pylori* proteins involved in reducing metronidazole, and the contribution of each of these proteins to metronidazole resistance is likely variable in different strains, making it more difficult to develop a reliable molecular method determining metronidazole susceptibility.

In a recent study (32), fluorescence resonance energy transfer-based real-time PCR performed on bacterial DNA isolated from pure culture was used to detect point mutations in the *gyrA* gene, which confers resistance to ciprofloxacin in *H pylori*. This assay appears reliable when compared with the E-test in 100 *H pylori* isolates (32). However, the sensitivity and specificity will need to be evaluated in the clinical setting, preferably using biopsy specimens.

The presence of amoxil resistance in *H pylori* is rarely reported. A single amino acid substitution in HP0597, a penicillin-binding protein 1A homologue of *Escherichia coli*, was demonstrated in an amoxil-resistant strain of *H pylori* (33). Similar to metronidazole resistance, there likely are other proteins involved in conferring amoxil resistance. The mechanism of amoxil resistance is still not well understood. Currently, a molecular diagnostic test is not available for the detection of amoxil resistance.

PCR-based assays have been developed to detect a restriction length polymorphism associated with a triple base pair substitution (adenine-guanine-adenine [926-928]/thymine-thymine-cytosine) in the 16S rRNA gene (34). This substitution is associated with tetracycline resistance (34); however, the accuracy of this mutation in determining tetracycline resistance is unknown.

Virulence genotyping

Numerous PCR methods (real-time PCR, PCR-length polymorphism, PCR-restriction fragment length polymorphism and line prop assay) have been used to determine the presence of *H pylori* virulence genes such as *cagA*, *cagE*, *vacA*, *babA* and *iceA* in fresh and paraffin-embedded tissues, or from DNA isolated from bacterial culture (35). These virulent genotypes have been associated with a more severe disease outcome; however, these results are debatable (36). The relevance of virulent genotyping in clinical practice remains to be determined.

CONCLUSIONS

One of the primary indications for upper GI endoscopy in children is the presence of persistent and severe upper abdominal symptoms. Upper GI endoscopy with mucosal biopsies remains the gold standard for the detection of *H pylori* infection and its mucosal complications in children. Molecular methods can be employed using obtained gastric biopsies to determine the presence of *H pylori* infection, antibiotic resistance and *H pylori* virulence factors. In the future, it is likely that these molecular methods will become more widely used in the clinical setting and allow for individualized treatment.

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