DOUGLASS HANLY MOIR PATHOLOGY

Quality is in our DNA

Doctors' Newsletter

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This is the third Doctors' Newsletter for 2017 and, once again, it is my pleasure to introduce it.

The articles included in each issue are on subjects which directly relate to one or other of the pathology sub-specialties and aim to have relevance and hold interest to a broad clinical readership. It is axiomatic, however, that when it comes to relevance and interest the readership is better placed to make that call. As I thank the contributors, may I take the opportunity to emphasise that we welcome any suggestions you may have of topics for inclusion in future issues.

Dr Kym Mina has been one of the most enthusiastic contributors to this Newsletter and in this issue, she discusses preconception carrier screening. Microbiology has also featured prominently and we express our gratitude to both Dr Ian Chambers and Dr Michael Wehrhahn for their review of the divergent arguments surrounding the clinical significance of *Dientamoeba fragilis*. The second microbiological article focuses on *Helicobacter pylori* – the only bacterium to have been classified by WHO as a human carcinogen. Finally, we thank Dr Jonathan Blackwell for his summary of blood group antibody testing in pregnancy.

I hope you enjoy what is likely to be the final Newsletter for 2017. Thank you for your continuing support for the practice and may I wish you and your loved ones an enjoyable holiday season and the very best for 2018.

Dr Colin Goldschmidt
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Cover: Red blood cells

Community Conscience

Douglass Hanly Moir Pathology (DHM) is a provider of pathology services to clinicians, hospitals and other individuals and institutions in NSW and beyond. Under a model and a culture of Medical Leadership this has been our core activity over the decades since the practice began and remains the case in 2017.

While this commitment to providing world-class diagnostic pathology services remains at our core, a comprehensive account of who we are in 2017, and what we do, requires many more words and much more space. The core itself has expanded, with the advent of new sub-disciplines such as Molecular Genetics and an ever-expanding range of tests of ever-increasing complexity in all others. As important, however, are the qualitative changes in the nature of the practice which have seen our role expand well beyond the circumscribed field of pathology testing.

As individuals, we are expected to be responsible citizens and while paying taxes and keeping the music down are deemed necessary elements of this, arguably, they are not sufficient. A range of other behaviours is encouraged, which reflect a broader set of ethical obligations to the community in which we live, for example recycling, conserving and avoiding sweat-shop brands.

Similar principles apply on a collective level. DHM regards social responsibility, including charitable giving, as a vital element in its definition as a practice. We are accountable to a range of communities at local, national and global levels. The Catalyst program is demonstration that we take this accountability seriously.

Catalyst forms the cornerstone of our giving program, acknowledging our social obligations and working to fulfil them in a meaningful and measurable way. Over the last 10 years, we have helped to create medical self-sufficiency for a number of communities in need, positively impacting the lives of tens of thousands of people. The list below outlines some of the projects in which we have been involved.

In 2017, our responsibilities extend beyond maintaining professional standards and ensuring good governance; we must also acknowledge responsibility in environmental and social spheres. To be sustainable we must be performing at a high level in each of these and our performance in each is a matter of public record.

As the bar continues to rise, we continue to rise to meet it. This gives me confidence as we move forward to meet the challenges of the future.

CATALYST PROGRAM		
Hospital	Region	
HEAL Africa	Goma, Democratic Republic of Congo	▪ Installation of pathology laboratory and radiology department ▪ Ongoing supplies ▪ Training of staff, including training of the first fully qualified pathologist ▪ Provision of teaching and other non-medical items
Fistula Hospital	Addis Ababa, Ethiopia	▪ Installation of pathology laboratory ▪ Training of staff ▪ Ongoing support
Barbara May Foundation Maternity Hospital	Mille, Ethiopia	▪ Medical and surgical equipment ▪ Planned installation of pathology laboratory (equipment and supplies) ▪ Staff training
Vision Maternity Centre	Bahir Dar, Ethiopia	▪ Medical and surgical equipment ▪ Planned installation of pathology laboratory (equipment and supplies) ▪ Staff training
His House of Hope Hospital	Yei, South Sudan	▪ Medical and surgical equipment ▪ Planned installation of pathology laboratory is now on hold due to the civil war

Dientamoeba fragilis: The Burden of Proof

Dientamoeba fragilis is a protozoan organism which is regularly found in the human gastrointestinal tract and, when it is found, an equally regular cause of debate over its clinical significance.

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Microbiologist

Historically, *D. fragilis* was believed to be a commensal but over time this changed and it became accepted as a potential cause of gastrointestinal disease. However, in a bout of PCR-prompted revisionism, the mood has swung back to *D. fragilis* being non-pathogenic. Such mood swings are unfortunate because they create the impression that pathogenicity is determined by opinion poll rather than objective evidence. This article is an effort to filter what has become a very muddy puddle.

Case Report: A nine-year-old boy with diarrhoea

The patient

A nine-year-old boy, Jack, presents (with his mother) to his GP in suburban Sydney. For the past two weeks, he has been experiencing watery diarrhoea, the onset occurring shortly after a week-long holiday in Fiji. There have been up to six loose motions per day, without blood or mucus, and otherwise he has been well. There has been no vomiting, abdominal pain or fever, his appetite is unchanged and there has been no weight loss. No other family member has had a similar illness.

On examination, the abdomen is soft and non-tender with no organomegaly. There is mild excoriation around the anus. Weight and height are tracking along the 50th and 75th centile for age, respectively.

Investigations

The GP requests the examination of a stool sample by microscopy, culture and PCR.

Investigations for common bacterial and viral gastrointestinal pathogens should be undertaken and a sample of unpreserved stool (brown top container) and a sample in SAF preservative (yellow top tube) should be submitted for microscopy, culture and PCR.

Conditions such as coeliac disease, lactose intolerance, gastro-oesophageal reflux, functional abdominal pain and urinary tract infection should also be considered and excluded, if necessary. In adults it is particularly important that a persistently altered bowel habit is not attributed to *D. fragilis* without considering the need for colonoscopy to exclude colorectal neoplasia.

Results

The specimen was unformed, there was no blood or mucus and no ova/cysts/parasites were seen on microscopy. Bacterial culture was negative for Salmonella, Shigella and Campylobacter. The faecal parasite PCR was negative for *Giardia intestinalis* and *Cryptosporidium parvum* but positive for *Dientamoeba fragilis*.

What is *Dientamoeba fragilis*? What is its significance? How should it be managed?

Dientamoeba fragilis is a protozoan parasite, most closely related to *Trichomonas* species. While this statement is uncontroversial, few others are. The potential for confusion begins with its taxonomy; despite the name, it is not an amoeba, and despite the absence of any external flagellum, it is classified as a flagellate. After this unpromising start, certainty and clarity recede further, to the point of opacity when a consensus view on clinical significance is sought.

Notwithstanding some thoughtful advice from the Royal College of Pathologists of Australasia to simply stop testing for *D. fragilis*, it is our opinion that turning a blind eye is not the best way forward.

There was once a general acceptance that *D. fragilis* was a potential pathogen and many authoritative references still take that position. In fact, under-diagnosis was widely thought to be more of a problem than over-diagnosis. But now that PCR is being used to detect the organism, it is being found and reported so often that its plausibility as a pathogen has suffered. Just as difficulty of detection is not a determinant of pathogenicity, however, ease of detection does not define commensalism. The problem of *D. fragilis* cannot be made to go away, nor can an adequate evidence-base be made to appear. However, until there is an evidence-base to support alternatives, advice and management must be based on the few accepted facts, supplemented by balanced opinion and reasoned speculation.

Routinely collected, unpreserved stool is an insensitive method of detection because *D. fragilis* has no easily-identifiable cyst phase and trophozoites degenerate rapidly unless the stool sample is preserved. Sodium acetate-acetic acid-formalin (SAF) is usually used for this purpose. In a recent study in our laboratory, *D. fragilis* was observed on routine microscopy in only 0.02% of submitted stool samples in contrast to 17% from either SAF preserved samples or by PCR.

The introduction of multiplex PCR assays for faecal pathogens has therefore released the genie from the bottle and now we are seeing *D. fragilis* reported much more often. For example, the rate of PCR-positive samples in children with gastrointestinal symptoms is up to 70%, and most case-control studies have found no over-representation of *D. fragilis* in children with diarrhoea or chronic abdominal pain, compared to those who are asymptomatic.

It should be emphasised that the detection of *D. fragilis* is not proof that it is causing disease. PCR cannot distinguish viable from non-viable organisms and it is possible that pathogenic and non-pathogenic sub-types exist and that PCR detects both. However, case reports of symptomatic individuals who have *D. fragilis* in their stool, who are treated and whose symptomatic improvement correlates with elimination of the parasite, are too numerous to exclude the possibility that *D. fragilis* is a human pathogen. More investigation is required.

The presence of these organisms in formed faeces, in asymptomatic individuals or people with vague symptoms (non-diarrhoea) is of unknown significance and, in most circumstances, treatment is not indicated. Samples positive for *D. fragilis* are also commonly positive for other parasites of questionable clinical significance, e.g. *Blastocystis hominis*. Current *D.fragilis* PCR assays may also cross-react with closely related animal parasites which are not suspected of causing disease in humans.

What should Jack's GP do when confronted with the report of *D. fragilis*?

It is important that clinicians and patients (and parents) appreciate the uncertain significance of a positive PCR result for *Dientamoeba fragilis*.

Given these uncertainties, the decision to treat *D. fragilis* must be based on more than its presence in stool. The highest quality evidence to date, a double-blind randomised controlled trial of 96 children, showed no change in gastrointestinal symptoms after a 10-day course of metronidazole. While PCR positivity was decreased in the treatment group 14 days post-therapy, there was no significant difference between the treatment and placebo groups at eight weeks post-therapy. This is evidence against metronidazole monotherapy. Other treatments (e.g. paromomycin), either alone or in combination with metronidazole, may be more effective but the clinical evidence is not strong.

In Jack's case, if symptoms persist and if no alternative diagnosis is found, treatment with a 10-day course of high dose metronidazole (15 mg/kg/dose, up to 600 mg) is an option to consider. Jack is over 8 years old and therefore doxycycline (100 mg bd) is also an option.

Paromomycin (via a compounding pharmacy or via SAS) may be a consideration in the setting of poor tolerance, contraindication to first line agents or incomplete response or relapse.

References

- Stark D et al, *Dientamoeba fragilis*, the neglected trichomonad of the human bowel. *Clin Micro* 2016; 29: 553–580

This article owes much to a Case Report authored by Dr James Newcombe (Clinical Microbiologist and Infectious Diseases Physician) and published in *Australian Doctor* (13 June, 2017).

Blood Group Antibody Testing in Pregnancy

The blood group and antibody screen detects and helps prevent haemolytic disease of the newborn (HDN). The most common maternal allo-antibody is anti-D and this is predicted by knowing the Rh blood group. Knowing the Rh blood group may lead to passive immunisation to prevent HDN. Other maternal allo-antibodies are detected by the antibody screen. Detection of significant allo-antibodies may allow treatment of a fetus at risk of anaemia or stillbirth.

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Haemolytic disease of the fetus and newborn (HDN) occurs when a mother forms allo-antibodies against an antigen present on the fetal red blood cells (RBC). The mother is negative for the antigen. The maternal antibodies cross the placenta and cause immune destruction of the fetal RBC. Only IgG antibodies cross the placenta and therefore IgM antibodies can be ignored in this context.

HDN may lead to fetal anaemia, oedema, ascites, heart failure, hepatosplenomegaly and stillbirth or neonatal anaemia and jaundice. Allo-antibodies that may be associated with HDN include anti-D, anti-c, anti-E and anti-K. About 1% of pregnancies will have a clinically significant antibody.

Causes of fetomaternal haemorrhage (sensitising events)

- Ectopic pregnancy
- Antepartum haemorrhage
- Miscarriage
- Abdominal trauma
- Termination of pregnancy
- External cephalic version
- Amniocentesis and other in-utero procedures
- Labour

Maternal allo-antibodies detected at DHM and BSP	
Most frequent	Other significant
D	K1
E	Ce
M	Fya
c	Jka

Testing for blood group antibodies in pregnancy is to prevent and detect HDN. This is done with an ABO and Rh blood group and antibody screen. All pregnant women should be tested with ABO and Rh blood group and antibody screen at the first prenatal visit (8–12 weeks). This screen should be repeated at 28 weeks. Another function of the blood group and antibody screen is to detect antibodies that may complicate transfusion support of the mother or neonate.

If the fetal blood cells have RhD antigens which the mother is lacking, the mother may form anti-D antibodies against the fetal RBC. If the antibody screen is positive, then further investigation is undertaken by the laboratory. This includes identifying the antibody and making an assessment regarding the strength of the antibody. If RhD antibody is detected, then the antibody level is titred in our laboratory and referred to the Australian Red Cross Blood Service (ARCBS) for quantification. A rising titre of anti-D or a moderate to high concentration of RhD antibody will require referral of the mother to a fetal medicine unit for further assessment and management.

If the mother is found to be RhD negative and has a negative antibody screen, then passive immunisation with anti-D globulin may prevent allo-immunisation. The RhD negative mother should be offered anti-D immunoglobulin at 28 and 34 weeks and at the time of delivery, or if there is a potential sensitising event, that is, fetomaternal haemorrhage (FMH). If the mother is to receive prophylactic anti-D, then the 28-week ABO and Rh blood group and antibody screen should be taken before anti-D administration.

Information regarding previous transfusion and pregnancy, and previous doses of anti-D, is important for interpreting the results of antibody testing and should be included with the request.

It is common to find low levels of anti-D in RhD negative mothers; knowing whether this is passively acquired from prophylactic anti-D or due to an immune response will depend on the history. If the history is not certain, it is presumed the anti-D is allo-immune and the mother will require appropriate follow-up.

Other Rh antibodies may be found and these may also lead to HDN, sometimes severe. With effective prophylaxis against HDN with passive anti-D immunoglobulin, other Rh antibodies have become proportionately more frequent. Anti-E or anti-c are the more likely antibodies. Anti-c is titred in our laboratories and quantified at ARCBS. Unlike anti-D, there are no globulin treatments available for other antigens.

Anti-K is another possible allo-antibody. This may occur as a result of transfusion or previous obstetric history. The severity of HDN due to anti-K is not predicted by the strength of the antibody and, therefore, early assessment by a fetal medicine specialist is recommended.

Blood group O mothers will have naturally occurring anti-A and anti-B. These are formed without FMH. Anti-A can cross the placenta to a fetus with blood group A or AB and lead to mild to moderate HDN, even in the first pregnancy.

The normal pregnant mother and fetus exchange red blood cells, but significant volumes of fetal red cells are found in the maternal circulation after FMH. The likelihood of FMH increases throughout the normal pregnancy and is universal after delivery.

The Kleihauer test is used after 20 weeks to assess the volume of FMH. Small volume FMH is most accurately detected by performing the Kleihauer test using flow cytometry techniques. A Kleihauer test should be performed on an RhD negative mother with a negative antibody screen within 72 hours of any sensitising event or delivery of an RhD positive baby.

A dose of anti-D should be given for all sensitising events. **Additional doses of anti-D may need to be given if the volume of FMH exceeds 6 mL.**

The formation of RhD allo-antibodies is proportional to the volume of FMH or volume of RhD incompatible blood transfused. Up to 15% of RhD negative women will form anti-D allo-antibodies after completing a pregnancy with an RhD positive neonate (if passive Rh immunoglobulin is not used). Thirty per cent of RhD negative women will never form RhD allo-antibodies and the reason for 'non-responders' is not known. ABO mismatch between mother and fetus (for example, O RhD negative mother with an A, B or AB RhD positive neonate) results in a nine-fold decrease rate of allo-immunisation.

It is reasonable to consider ABO and Rh or other relevant blood group typing of fathers if an antibody is detected by the antibody screen.
Rarely, RhD positive mothers express a very weakened form of RhD antigen (Category VI) that may lead to allo-immunisation. The presence of Category VI RhD will be indicated in the test comments.

Useful references

- RANZCOG, Guidelines for the use of Rh(D) Immunoglobulin (Anti-D) in obstetrics in Australia, https://www.ranzcog.edu.au/ (Keywords 'RhD immunoglobulin, obstetrics')
- National Blood Authority Australia, Guidelines on the prophylactic use of Rh D immunoglobulin (Anti-D) in obstetrics, https://www.blood.gov.au/system/files/documents/glines-anti-d.pdf (Keywords 'Guidelines Anti-D')
- Australia Red Cross Blood Service, Anti-D prophylaxis, https://transfusion.com.au/disease_therapeutics/fetomaternal/HDN
- Australian Red Cross Blood Service, Transfusion Resources Centre, http://resources.transfusion.com.au/ (Keywords 'RhD support material')

Tests referred to in this article

ABO and Rh blood group and antibody screen

Test in first trimester and 28 weeks. Further testing as recommended based on the results.

Kleihauer test

Used to quantify the amount of FMH if there is a potential sensitising event after 20 weeks and at the time of delivery in an RhD negative mother.

Preconception Carrier Screening

Preconception carrier screening can identify individuals or couples at high risk of having a child with a serious heritable disorder. This test is becoming an essential part of prenatal care planning. It allows patients the opportunity to explore their reproductive options, and helps ensure they can make properly informed decisions.

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The importance of preconception carrier screening

Preconception carrier screening provides information on the carrier status of your patients for three of the most common heritable disorders in Australia:

- Cystic fibrosis (CF)
- Spinal muscular atrophy (SMA)
- Fragile X syndrome (FXS)

In the absence of screening, the combined incidence of these disorders at birth is comparable to the incidence of Down syndrome. Preconception carrier screening enables patients to reduce that risk. Current guidelines* recommend that screening for common genetic disorders, including CF, SMA and FXS, may be offered to all women. It is also recommended that individuals with an increased likelihood of carrier status based on ethnicity be offered screening for haemoglobinopathies.

CF and SMA are autosomal recessive disorders and so, in many cases, individuals are unaware that they are carriers. Screening for FXS is particularly important if there is a family history of intellectual disability, but is also suitable for women with population-level carrier risk.

*(Prenatal screening and diagnosis of chromosomal and genetic conditions in the fetus in pregnancy (C-Obs59), RANZCOG College Statements and Guidelines)

Condition	People with the condition	Carriers of the condition
CF	1 in 3,000 births	1 in 25
SMA	1 in 6,000–10,000 births	1 in 35
FXS	1 in 7,000–11,000 people	1 in 250

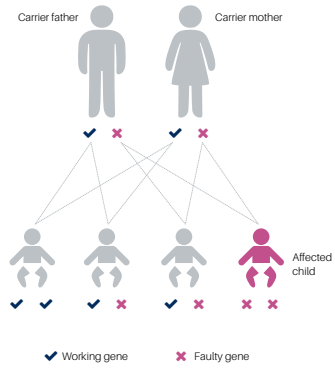
Information for testing

Preconception carrier screening identifies carriers by testing for mutations that cause most cases of CF, SMA and FXS. It is a DNA-based test performed on a standard blood draw that can be collected at any Douglass Hanly Moir Pathology collection centre. Thalassaemia screening by HbEPG can also be requested on the same collection.

Testing can be performed on individuals or couples. There are two options for carrier testing of couples:

- A sample is collected and tested from the female partner first. If she is found to be a carrier of CF or SMA, a sample can be collected from the male partner for carrier testing for the same disorder. Testing of the male is not required for FXS.
- Samples are collected from both partners and tested. The advantage of this is that in the event of a positive screen, anxiety while waiting for the second can be avoided.

The best time to establish carrier status is prior to conception, however screening can still be performed in early pregnancy. Family history can modify carrier risk assessment and interpretation of genetic test results. It is therefore essential that any details regarding family history, including relationship to patient and previous genetic test results, are noted on the request, along with pregnancy status if applicable.

Preconception screening identifies carriers by testing for mutations that cause most cases of CF, SMA and FXS		
The disorder	**The test**	**The risk**
Cystic fibrosis (CF) is the most common inherited disorder in Caucasians. It affects respiratory and gastrointestinal function, resulting in progressive lung disease, recurrent respiratory tract infection, pancreatic insufficiency and male infertility.	50 common mutations in the CFTR gene, responsible for >85% of cases of CF in the Australian population.	A couple can only have a child with CF or SMA if they are both carriers. When both partners are carriers, they have a 25% risk of having an affected child. 
Spinal muscular atrophy (SMA) is the most common genetic cause of mortality in children under two. It is characterised by progressive symmetric muscle weakness and atrophy that can be complicated by respiratory, orthopaedic and nutritional comorbidities.	Deletions of the SMN1 gene, responsible for 96% of cases of SMA.	
Fragile X syndrome (FXS) is the most common form of inherited intellectual disability, developmental delay and behavioural abnormalities, including autism.	Expansions of the CGG triplet repeat region of the FMR1 gene, responsible for 99% of cases of FXS.	Female carriers are at 50% risk of passing the mutation to their children. The risk of having a child with FXS depends on the size of the expansion. Male and female carriers of premutations are at increased risk of tremor-ataxia syndrome and premature ovarian insufficiency (women).

Results

Results are typically available within two weeks of sample collection. The result provided will indicate whether a mutation was found, and the implication for the patient.

Result	Interpretation
Carrier for CF or SMA	Individual is at increased risk of having affected children. Testing of reproductive partner is recommended. Genetic counselling is recommended if both partners are carriers.
Carrier for FXS (females)	Individual is at increased risk of having affected children. This result also has potential medical implications for the individual being tested. Genetic counselling is recommended.
Carrier for FXS (males)	Carrier testing of males should be considered carefully. Male mutation carriers are not considered to be at risk of having children with FXS; however, their daughters will inherit a premutation and be at risk of having affected children themselves. This result also has potential medical implications for the individual being tested. Genetic counselling is recommended.
Carrier status for CF, SMA and FXS unlikely	A mutation was not detected but the possibility that the patient is a carrier cannot be excluded.

Please note that preconception carrier screening is a test of common mutations in certain genes. Our clinical and scientific experts have selected the most common mutations and best technology available to detect the majority of the relevant mutations for these three inherited conditions.

The test does not detect every mutation that could cause CF, SMA and FXS, or mutations in other genes responsible for other disorders. If no mutation is found, the risk of the patient being a carrier is greatly reduced, but the possibility is not eliminated. The reduction in risk varies according to the patient's ethnicity; please contact the laboratory for details.

Depending on the results provided, referral for genetic counselling and discussion of reproductive options may be appropriate. A list of private and public providers of clinical genetics and genetic counselling services is available from Sonic Genetics on request or visit our website, www.sonicgenetics.com.au/doctors/resources.

References

- Cystic Fibrosis Federation Australia, www.cysticfibrosis.org.au (Accessed September 2017)
- The Fragile X Association of Australia, www.fragilex.org.au (Accessed September 2017)
- Spinal Muscular Atrophy Australia, www.smaaustralia.org.au (Accessed September 2017)

Helicobacter pylori & Gastric Cancer

Helicobacter pylori is currently the only bacterium which has been categorised as a carcinogen by the WHO International Agency for Research on Cancer. The link between viral infection and cancer development is well-established and is the basis for several population-based screening and prevention programs. *H. pylori* may be the bacterial prototype for similar public health interventions. While the available evidence does not support widespread testing for, and eradication of, *H. pylori* in asymptomatic individuals, its global prevalence and known disease-associations make it a human pathogen of evolving importance.

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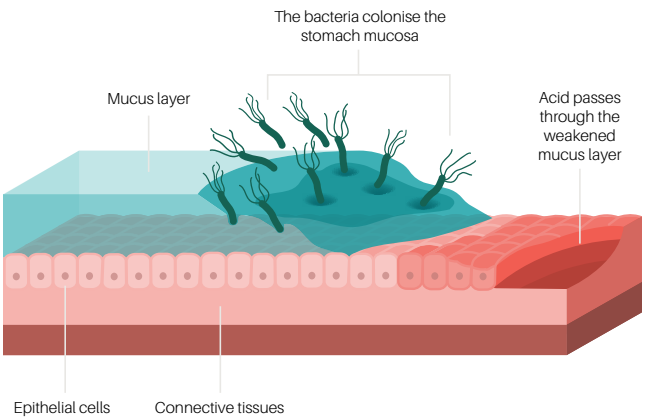
Epidemiology and disease association

Since its recognition as a potential human pathogen in 1982, *Helicobacter pylori* has been implicated as a cause of chronic gastritis and peptic ulcers. It has been estimated to affect up to two thirds of the world's population, occurring at any age and across all socioeconomic groups, although the risk of acquisition is related to socioeconomic status and living conditions early in life.

H. pylori is transmitted via contaminated food and water and by direct mouth-to-mouth contact. Once acquired, infection persists, but in most people does not cause illness. However, the high prevalence of infection and the well-established link between infection and gastric cancer gives *H. pylori* a significance which warrants wider recognition.

H. pylori and gastritis



The organism

H. pylori is a spiral-shaped, Gram-negative bacterium which, while slow-growing, can be isolated readily in culture. Its most notable metabolic characteristic, and the basis for two widely used tests for the presence of *H. pylori* in the stomach, is the abundant production of the enzyme urease. There are more than 30 other species in the genus helicobacter; non-pylori *Helicobacter* species have been detected in humans and have been occasionally associated with gastritis and peptic ulcer disease.

H. pylori and gastric cancer

Gastric cancer is the fourth most common cancer, worldwide, and the second most common cause of cancer-related deaths. It is less common in Western societies than it is in Asia and South America and infection with *H. pylori* is the primary identified cause. However, the association between *H. pylori* and cancer is complex; there is an increased risk of non-cardia gastric adenocarcinoma but also the possibility of reduced risk of carcinoma involving the cardia and the oesophagus.

- **Gastric adenocarcinoma:** Individuals infected with *H. pylori* have a risk of gastric adenocarcinoma (non-cardia) approximately eight times that of uninfected individuals.
- **MALT lymphoma:** This is a type of non-Hodgkin B-cell lymphoma affecting the lining of the stomach. Gastric mucosa usually lacks lymphoid tissue but its development is sometimes stimulated by *H. pylori* colonisation. This tissue can give rise to mucosa-associated lymphoid tissue (MALT) lymphoma and nearly all patients with this diagnosis are infected with *H. pylori*.

What is the mechanism behind the oncogenic potential of *H. pylori*?

There is a complex interplay between *H. pylori* and other components of the gastric microbiota, as well as host genetic polymorphisms and dietary factors, which determine the path to gastric cancer. An association has been made between cagA-mediated cytotoxin production, inactivation of tumour suppressor proteins and oncogenesis. Only cagA-positive strains of *H. pylori* have been associated with gastric cancer. More recent evidence has established a link between chronic *H. pylori* infection, unremitting stem cell proliferation and carcinogenesis.

Does eradication of *H. pylori* reduce gastric cancer rates?

The role of *H. pylori* in the causation of gastric cancer raises the possibility of cancer prevention through screening and eradication. Long-term follow-up of patients who have received eradication treatment for *H. pylori* has been associated with a significantly higher rate of regression of precancerous lesions and a reduction in the incidence of gastric cancer.

Diagnosis of *H. pylori* infection

1. Endoscopy and biopsy

The diagnosis of *H. pylori* can usually be established by endoscopy by one of three methods: direct urease testing, histology and bacterial culture.

- **Biopsy urease testing:** Antral biopsies can be tested for urease activity during the procedure, with a diagnostic sensitivity and specificity of approximately 95%.
- **Histology:** Histology of gastric biopsies not only detects *H. pylori* infection but also allows the diagnosis of associated gastritis, intestinal metaplasia and MALT lymphoma. While sampling variability may lead to occasional false-negatives (hence the recommendation for multiple biopsies), the diagnostic accuracy of histology is >95%.
- **Bacterial culture and sensitivity testing:** Rarely used for diagnosis but may guide antibiotic selection in cases of failed therapy.

2. Non-invasive methods (not requiring endoscopy)

These include urea breath testing (UBT), stool antigen testing and serology.

- **Urea breath testing:** A labelled carbon isotope is given by mouth; *H. pylori* liberates tagged CO2 which can be detected in breath samples.
  There are two types of UBT, one using a 14C isotope and the other 13C, each with similar diagnostic accuracy. Limited availability of 13C UBT has meant that the 14C UBT is most widely used. The dose of radiation in the 14C test is minimal (approximately 3 microSv) and equivalent to half a day's exposure to environmental radioactivity, such as sunlight. At such levels, there is no theoretical reason for the test's unsuitability.
  The sensitivity and specificity of UBT are approximately 90–95% and 95–100%, respectively. False-negative results may occur in patients who are taking proton pump inhibitors (PPIs), bismuth or antibiotics. To reduce false-negative results, the patient should be off antibiotics for at least four weeks and off PPIs for at least one week. PPIs can be switched to H2 receptor antagonists until about six hours prior to the test.
- **Stool antigen assay:** *H. pylori* antigen is present in the stool of individuals with gastric colonisation and its detection is a sensitive and specific method for diagnosis. This approach is of particular use in patients who are unable (due to age or disability) to cooperate in performing a UBT. It is also the best alternative in patients who cannot fast for the required six hours before a breath test. The same requirement for antibiotic and PPI withdrawal prior to testing applies to antigen detection as it does to UBT.
- **Serology:** Detection of IgG antibodies to *H. pylori* has high sensitivity for past or current infection but cannot reliably distinguish between them. It is not recommended, either for routine diagnosis of infection or follow-up of therapy.

Confirmation of eradication

Confirmation of eradication should be considered for all patients receiving treatment for *H. pylori* because of the availability of accurate, non-invasive tests (UBT and stool antigen) and because of increasing antibiotic resistance.

Urea breath testing performed at least four weeks after treatment has been promoted as the test of choice to confirm eradication of infection. Stool antigen testing is an alternative where UBT is not available or not appropriate. Antibiotics and bismuth should be discontinued for at least four weeks and PPIs at least one week prior to testing.

Medicare rebate

The UBT is covered by Medicare for the confirmation of *H. pylori* colonisation and monitoring of the successful eradication of *H. pylori.*

References

- Wroblewski LE, Peek RM, Wilson KT. *Helicobacter pylori* and Gastric Cancer: Factors That Modulate Disease Risk. *Clinical Microbiology Reviews*. 2010; 23(4):713-739

DOUGLASS
HANLY MOIR
PATHOLOGY

Our Doctors' Newsletters contain articles written by our pathologists which focus on current issues and recent developments in pathology. Suggestions from you, which we invite wholeheartedly, are the best guarantee that our Doctors' Newsletter becomes a resource of maximum possible interest, information and relevance. If you have any topics you would like to suggest please feel free to contact Dr Ian Chambers (Medical Editor, DHM Publications) at med.ed@dhm.com.au.

Please note, this Newsletter can also be viewed on our website via the Clinician Publications link.

We look forward to hearing about your topics of interest and encourage your participation.

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Medical Editor

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