Helicobacter pylori: From its discovery to a revolution in gastroenterology

Y. Glupczynski

Laboratoire de Microbiologie
Cliniques Universitaires UCL de Mont-Godinne
Peptic ulcer and Nobel prize...over more than a century

- The presence of HCl in the stomach by W. Prout in 1823
- Vagus plays crucial role in gastric HCl secretion by J.P. Pavlov in 1895
- Gastrin is gastric HCl stimulant by J.S. Edkins in 1905
- Dictum; NO ACID NO ULCER by K. Schwartz in 1910
- Histamine as gastric HCl secretagogue by L. Popielski in 1916
- H₂-receptor antagonists by J.W. Black in 1972 and proton pump inhibitors by G. Sachs in 1980 showing strong inhibition of HCl secretion stimulated by various secretagogues and anti-ulcer efficacy

*Fig. 1. Historical background; major discoveries in gastrology (star indicates Nobel prize)*
**H. pylori**: Some key dates

<table>
<thead>
<tr>
<th>Microscopic observation of spiral-shaped bacteria in stomach</th>
</tr>
</thead>
<tbody>
<tr>
<td>1893 Bizzozero animal</td>
</tr>
<tr>
<td>1906 Krienitz human</td>
</tr>
<tr>
<td>1975 Steer human</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982 Marshall human</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982 Marshall <em>Campylobacter pyloridis</em></td>
</tr>
<tr>
<td>1989 Goodwin <em>Helicobacter pylori</em></td>
</tr>
</tbody>
</table>
Evolution of Number of publications on *H. pylori* and gastroduodenal diseases

N = > 22,000 articles listed in PubMed
The milestone publications...

The Lancet • Saturday 16 June 1984

UNIDENTIFIED CURVED BACILLI IN THE STOMACH OF PATIENTS WITH GASTRITIS AND PEPTIC ULCERATION*

Barry J. Marshall  J. Robin Warren
Departments of Gastroenterology and Pathology, Royal Perth Hospital, Perth, Western Australia

Summary  Biopsy specimens were taken from intact areas of antral mucosa in 100 consecutive consenting patients presenting for gastroscopy. Spiral or curved bacilli were demonstrated in specimens from 58 patients. Bacilli cultured from 11 of these biopsies were gram-negative, flagellate, and microaerophilic and appeared to be a new species related to the genus Campylobacter. The bacteria were present in almost all patients with active chronic gastritis, duodenal ulcer, or gastric ulcer and thus may be an important factor in the aetiology of these diseases.

H. Pylori on gastric epithelial cells

Warren JR, Marshall BJ; Lancet i: 1273-5; 1983
Fruitless attempts to culture the elusive organism until …

Culture of gastric mucosa biopsies from 34 patients negative (after 48 h)

First cultures became positive only after prolonged Easter week-end
Resistance from the medical establishment…

Submission to the 1983 meeting of the Australian Gastroenterological Society was rejected.…

…but was accepted to the 2nd International Microbiology Workshop on *Campylobacter* infections in Brussels in 1983
Attempts to fulfil Koch’s postulates (I) ...

Self ingestion of a pure culture of *H. pylori* (10⁹ organisms)

- Day 5: halitosis, nausea, vomiting of acid free gastric juice
- Day 10: Endoscopy with biopsy
  Acute gastritis with many *H. pylori*
- Day 14: Rx with Bismuth/tinidazole
  resolution of symptoms

Attempts to fulfil Koch’s postulates (II)...

PROSPECTIVE DOUBLE-BLIND TRIAL OF DUODENAL ULCER RELAPSE AFTER ERADICATION OF CAMPYLOBACTER PYLORI

BARRY J. MARSHALL1 C. STEWART GOODWIN2
J. ROBIN WARREN3 RAYMOND MURRAY4
ELIZABETH D. BLINCOW3 STEPHEN J. BLACKBOURN3
MICHAEL PHILLIPS5 THOMAS E. WATERS5
CHRISTOPHER R. SANDERSON6

Departments of Gastroenterology,1 Microbiology,1 Histopathology,1
and Pharmacy,3 Royal Perth Hospital, Perth, Western Australia;
and Centre for Advanced Studies in Health Sciences, Curtin
University, Perth6

Summary 100 consecutive patients with both duodenal ulcer and Campylobacter pylori
infection were followed up to see whether eradication of C pylori affected ulcer healing or relapse. Patients were
randomly assigned to 8 weeks of treatment with cimetidine or colloidal bismuth subcitrate (CBS), with tinidazole or
placebo being given concurrently from days 1 to 10, inclusive. Endoscopy, biopsy, and culture were done at
entry, in weeks 10, 22, 34, and 62, and whenever symptoms recurred. There was no maintenance therapy. C pylori
persisted in all of the cimetidine-treated patients and in 95% of those treated with cimetidine/tinidazole, but was
eradicated in 27% of the CBS/placebo group and 70% of the CBS/tinidazole group. When C pylori persisted, 61% of
duodenal ulcers healed and 84% relapsed. When C pylori was cleared 92% of ulcers healed (p < 0.001) and only 21%
relapsed during the 12 month follow-up period (p < 0.0001).

Cimetidine vs Bismuth (CBS) (8 Wks) + Placebo or tinidazole (10d)

Eradication of H. pylori:
increases healing of duodenal ulcer
92% (Hp-) vs 61% (Hp+)
decreases relapse rates at 12 months
21% (Hp-) vs 84% (Hp+)

Marshall BJ; Lancet 1988; 332;: 1437-42
Hill causality criteria for association between *H. pylori* infection and gastroduodenal ulcer

- Strong association
- Temporal relationship
- Major effects of therapeutic intervention on outcome
- Biologic plausibility
- Biologic gradient?
- But, no specificity of association
**H. pylori** : Epidemiology

- **Chronic infection**
- **Prevalence increases with age**
  - incidence = 0.5-1% by person / year
- **Infection acquired earlier in life and increased prevalence**:
  - developing countries
  - populations with low socio-economic status
- **Inter-human transmission**
  - oral-oral and/or faecal-oral
Prevalence of *H. pylori* infection with age

<table>
<thead>
<tr>
<th>Age range (years)</th>
<th>Developing countries</th>
<th>Developed countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>10-75%</td>
<td>75%</td>
</tr>
<tr>
<td>40-50</td>
<td>64-96%</td>
<td>80%</td>
</tr>
<tr>
<td>&lt;20</td>
<td>6-39%</td>
<td>7-54%</td>
</tr>
<tr>
<td>40-50</td>
<td></td>
<td>50%</td>
</tr>
</tbody>
</table>

Adapted with permission from Heatley- *Helicobacter pylori and Gastrointestinal Disease*; Oxford, UK: Blackwell Scientific Publications.
Divergent response to *H. pylori* infection

**Chronic *H. pylori* Infection**

- 10-15%
  - Antral predominant Gastritis
    - Acid & Gastrin, little or no atrophy
    - low risk of gastric Ca

- ~80%
  - Mild Mixed Gastritis
  - Normal Acid
  - No significant disease

- 2.5%
  - Corpus predominant Gastritis
    - MAG, Acid, Gastrin
    - high risk of gastric carcinoma

**Duodenal ulcer disease**

**Gastric Carcinoma**

**? Bacterial ? Environment ? Host**
### Major pathogenic factors of *H. pylori*

<table>
<thead>
<tr>
<th>Virulence factors</th>
<th>Function</th>
<th>Present in all strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urease</td>
<td>Acid resistance; Nitrogen metabolism, Escape to immunologic response</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>Cytotoxic effect</td>
<td></td>
</tr>
<tr>
<td>Flagella</td>
<td>Motility</td>
<td>Y</td>
</tr>
<tr>
<td>BabA</td>
<td>Adhesion to Lewis(^b) antigens</td>
<td>N</td>
</tr>
<tr>
<td>AlpA, AlpB</td>
<td>Adhesins (receptors not identified)</td>
<td>Y</td>
</tr>
<tr>
<td>Catalase</td>
<td>Detoxification (escape to immunologic response)</td>
<td>Y</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lewis(^x,y) antigens</td>
<td>Molecular mimicry (escape to immunologic response), adherence</td>
<td>N</td>
</tr>
<tr>
<td>(lipopolysaccharide)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VacA</td>
<td>Cytotoxicity</td>
<td>N</td>
</tr>
<tr>
<td>CagA</td>
<td>Immunodominant antigen, unknown function</td>
<td>N</td>
</tr>
<tr>
<td>Cag pathogenicity island</td>
<td>Secretion system (type IV)? Induction of inflammation</td>
<td>N</td>
</tr>
</tbody>
</table>
Cag pathogenicity island

Chromosome of H. pylori

Cag II region

Cag I region

IS605

TnpA TnpB

Censini et al., PNAS 1996, 93: 14648-53
Role of cag pathogenicity island and CagA protein

Rearrangements of cytoskeleton

Polymerisation of actin filaments

Induction of IL-8 secretion

Inflammation of gastric mucosa

Apoptosis

(Covacci et Rappuoli, 2000)
H. pylori genome
(Tomb et al. 1997, Alm et al. 1998)

Assign function to a putative genes
"H. pylori: from infection to gastric cancer"

- H. pylori
- Chronic active gastritis
- Interaction DNA/mutagene
- Atrophic gastritis
- Intestinal Metaplasia
- Dysplasia
- Gastric Cancer
H. pylori and gastric pathology

Pro-inflammatory cytokines gene polymorphisms
- **IL-1B-511*T**
- **IL-1-RN*2*2**
- **IL-10 ATA haplotype**
- **TNF-A-308*A**
- **IL-8-251*A**

Innate immune response gene polymorphisms
- **TLR4+896*G**

Bacteria promoted factors
- CagA, VacA
- Endotoxins (LPS)
- Growth factors, gastrins, COX-2-Prostaglandins
- Reactive oxygen species

Environmental factors
- Smoking
- Dietary factors
- Abuse of stimulants

H. pylori infection may be also responsible for gastric cancerogenesis that follows expression and action on gastric mucosa of cytotoxins (CagA and VacA), proinflammatory cytokines and gastrins.
June 1994

• Working group WHO/IARC

« Establishment of a definite link between H. pylori infection and gastric cancer in humans »

« H. pylori considered as Group 1 (definite) carcinogen »

IARC Monograph. - Evaluation of carcinogenic risks for humans - 1994, Lyon, France
**H. pylori and gastric cancer**

- Epidemiologic data
  - Geographic and temporal concordance in incidence

- Cross-sectional case-control studies
  - 50 -100% of gastric cancers infected with *Hp*

- Prospective nested case-control studies
  - *Hp* infection --> Risk of developing gastric cancer increased 2- to 9-fold

- Causal relationship in animal model
  - mongol gerbils
The only good *Helicobacter* is a dead *Helicobacter*!
Evolution of the understanding theories of gastroduodenal ulcers

No acid... No ulcer  
(K. Schwartz 1910)

H2-receptor antagonists  
(JW Black 1972)

Proton-pump inhibitors  
(G Sachs 1980)

No Helicobacter  
...No ulcer  
(B Marshall, 1988)
**H. pylori** infection: Therapeutic indications

- Peptic ulcer disease* – active or not
- Gastric MALT lymphoma
- Atrophic gastritis
- Post-gastric cancer resection
- First degree relatives of gastric cancer patients
- Patient’s wishes – after full consultation with their physician

Hp eradication Rx cost-effective*: > anti-H2 blockers for healing ulcers = to maintenance Rx for preventing recurrences) (1-2 yrs)

* Meta-analysis CRG, Ford, AJG 2004
Characteristics of optimal drugs for the treatment of *H. pylori* infection

- High in vitro antibacterial activity
- High concentration in gastric mucosa/mucus
- Active by endoluminal and systemic routes
- Stable over wide range of pH (<2-7)
- Good tolerance / few side effects
- Low propensity for resistance
- Inexpensive
# First choice antibiotics

<table>
<thead>
<tr>
<th>Clarithromycin</th>
<th>Amoxicillin</th>
<th>Metronidazole</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Green Smile" /></td>
<td><img src="image" alt="Green Smile" /></td>
<td><img src="image" alt="Green Smile" /></td>
</tr>
<tr>
<td>- Most active single agent</td>
<td>- No resistance development</td>
<td>- Activity not influenced by pH</td>
</tr>
<tr>
<td>- Good diffusion in gastric mucosa</td>
<td>- Activity not affected by acidic pH</td>
<td>- Clinical efficacy little affected by resistance</td>
</tr>
<tr>
<td>- Synergy with acid-suppressive agents</td>
<td>- Broad-spectrum</td>
<td>- Inexpensive</td>
</tr>
<tr>
<td>- Increasing resistance</td>
<td>- Side effects (gastrointestinal)</td>
<td>- Side effects (gastrointestinal)</td>
</tr>
<tr>
<td>- Decreased efficacy in areas of high resistance (20%)</td>
<td>- Side effects (gastrointestinal, dermatologic)</td>
<td>- High resistance rates in several areas (&gt;40%)</td>
</tr>
<tr>
<td>- High cost</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Second choice antibiotics

**Tetracyclines**
- Resistance rare
- Synergy with bismuth salts
- Clinical efficacy in quadruple therapy
- Inexpensive
- Side effects (gastrointestinal, rash)

**Fluoroquinolones**
- Potential interest for rescue therapy if previous treatment failures
- Well tolerated
- Wide usage of FQ in other indications
- Poorly active at acidic pH
- Resistance increasing
Proton-pump inhibitors

- Bacteriostatic activity against *H. pylori*

- Synergy with some antimicrobials (metro, clari, tetra)

- Effect of acid suppression (↑ gastric pH):
  - increases activity of several antibiotics (macrolides, quinolones)
  - Improved penetration of bismuth salts in gastric mucosa
  - Increased concentrations of metro, clari, tetra in the stomach

- Improve clinical efficacy of dual antimicrobial regimens
Adjuvant effect of Omeprazole on *H. pylori* eradication by amoxicillin

Unge, Bordeaux 1988
Therapeutic principles (1)

Rational choice of therapeutic agents

- Based on clinical experience
- Association regimens including:
  - amoxicillin
  - clarithromycin
  - 5-Nitroimidazoles
  - Tetracycline
  - Bismuth salts
- Other agents active in vitro have proven ineffective in vivo
Importance of drug associations

<table>
<thead>
<tr>
<th>Drug Association</th>
<th>% Eradication (ITT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mono (2-4 wks)</td>
<td>15</td>
</tr>
<tr>
<td>Bi (2 wks)</td>
<td>60</td>
</tr>
<tr>
<td>Tri (1 wk)</td>
<td>85</td>
</tr>
<tr>
<td>Quadri (1 wk)</td>
<td>88</td>
</tr>
</tbody>
</table>
Hp: Therapeutic principles (3)

Treatment duration: eradication rates with bismuth based triple therapies

% eradication (PP)

MTZ resistant

MTZ susceptible

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 days
Importance of resistance on Hp eradication

Mégraud, Gut 2004
Importance of patients compliance for eradication of *H. pylori*

![Graph showing eradication rates for duodenal and gastric ulcers.](image)

- **Duodenal ulcer**
  - Compliance >60%: 97%
  - Compliance <60%: 75%

- **Gastric Ulcer**
  - Compliance >60%: 91%
  - Compliance <60%: 33%

Graham, *Gastroenterol* 1992
### Factors of importance for predicting outcome of therapy in Hp infection

**PROVEN**
- Choice of agents
- Choice of associations
- Compliance
- Antimicrobial resistance
- Dosage
- Duration of therapy

**POSSIBLE**
- Drug formulation
- Number of daily administration
- Drug intake in relation to meal
- Polymorphism of CYP2C19 in metabolism of PPIs
First line therapy

1 week, Triple therapy, 2x/daily (PPI + 2 ABs)

- PPI standard dose bid (omeprazole, lanso, panto)
  + Clarithromycin 500 mg bid (C)
  + Amoxicillin 1000 mg bid (A)
  + or
  + Metronidazole 500 mg bid (M)

CA preferred in area with Metro-R > 40%

≥ 80% cure rates on ITT to basis

Malfertheiner, APT 2002
Primary resistance rates of *H. pylori* in Belgium

% resistance
(N=151, 10 centres)

<table>
<thead>
<tr>
<th></th>
<th>Metro</th>
<th>Clari</th>
<th>Cipro</th>
<th>Amoxi</th>
<th>Tetra</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30,6</td>
<td>13,6</td>
<td>18,1</td>
<td>1,3</td>
<td>0,7</td>
</tr>
</tbody>
</table>

*I see resistance coming*

Belgian Helicobacter Study Group, 2004
Antimicrobial resistance of *H. pylori* in Europe

**Risk factors for resistance**

**Metro-R**
- Born outside Europe (OR: 2.7)
- Living in East. Europe (OR: 1.9)
- Female (OR: 2.3)

**Clari-R**
- Living in South. Europe (OR: 2.3)
- Age < 12 yrs (OR: 1.8)

22 centres, 17 pays
N=1274 (N° d’isolats/Centre = 64, valeurs extrêmes: 21-115)

Glupczynski, EJCMID 2001
Consumption of macrolides in 17 European countries in 1998

www.ua.ac.be/ESAC
Secondary resistance of *H. pylori* after eradication failure

First Rx: PPI-AC (90% cases)

- % resistance (N=225)
  - Amox: 1
  - Clari: 64
  - Métro: 53
  - Clari + Métro: 37

*Mégraud et al. Gut 2001*
Second line therapy

Bismuth quadruple therapy, 10-14 d
(failure, allergy, intolerance, high level of resistance to clari)

PPI standard dose bid (omeprazole, lanso, panto)
+ Bismuth salt (CBS) 120 mg qid
+ Metronidazole 500 mg tid
  Tetracycline 500 mg qid
+ or
  Amoxicillin 500 mg tid

Unaffected by Clari-R; little influenced by Metro-R
Cost < to PPI-triple therapy

≥ 85% cure rates on ITT to basis

Fishbach, APT 2004
Rescue therapy

after failure of two courses of different therapies

Treatment adjusted on the basis of culture and susceptibility results (antibiogramme)

Should be handled on « Case-by-case » basis

Avoid re-using same agents (clari, metro)

Proposed triple therapies (under evaluation):

PPI + Amox + fluoroquinolones (Levoflo, Moxiflo)

rifamycins (rifaximin, rifabutin)
Evolution of *H. pylori* resistance

N=555 Hp isolates; 1989-2000

<table>
<thead>
<tr>
<th>Period</th>
<th>Clari</th>
<th>Metro</th>
<th>Clari + Metro</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989-1994</td>
<td>6.1</td>
<td>16.9</td>
<td>1.3</td>
</tr>
<tr>
<td>1995-2000</td>
<td>16.6</td>
<td>18.9</td>
<td>2.6</td>
</tr>
</tbody>
</table>

P < 0.001

Bontems et al., PIJD 2001
Evolution of prevalence of \textit{H. pylori} infection in Europe

\begin{figure}
\centering
\includegraphics[width=\textwidth]{chart.png}
\caption{Evolution of prevalence of \textit{H. pylori} infection in Europe}
\end{figure}
Perspectives

• *H. pylori* infection becoming more rare

• Consequences of infection in terms of burden of associated diseases (ulcer, cancer) is going to be with us for a while
The *H. pylori* saga ending in a Nobel Prize …

• …Being at the right place at the right time, and seeing what other people had seen but thinking what nobody else thought…

• The role of chance…

• … »Challenging established dogmas with passion, tenacity and prepared mind …

• Possibility of making important discoveries as clinicians in the course of daily practice…