

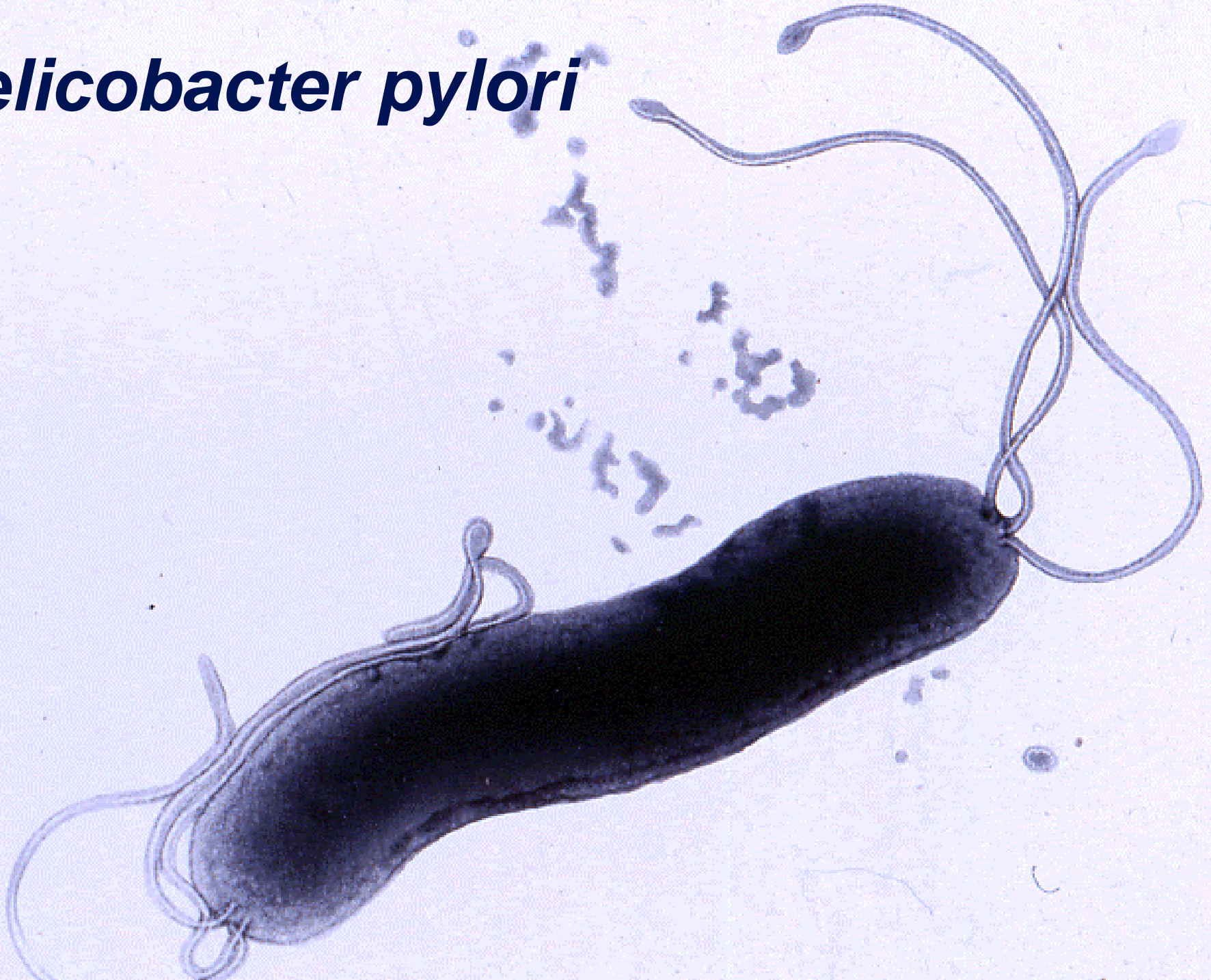
# ***Helicobacter pylori: pathogenesis of infection and management***

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***Helicobacter pylori***



A black and white photograph of two men, Barry Marshall and Robin Warren, sitting at a desk in a laboratory. Barry Marshall is on the left, wearing a white shirt and a striped tie, with his hand resting on his chin. Robin Warren is on the right, wearing a dark sweater over a white shirt and glasses, with a beard. The background shows bookshelves filled with books, a microscope, and other laboratory equipment.

**Barry Marshall**

**Robin Warren**

## Laureates of Nobel Prize in Medicine 2005



**Barry Marshall**

**Robin Warren**

# Discovery of *H. pylori*: a breakthrough in Gastroenterology

- **Change in concept**

Gastric diseases are now considered as Infectious diseases

- **Change in practice**

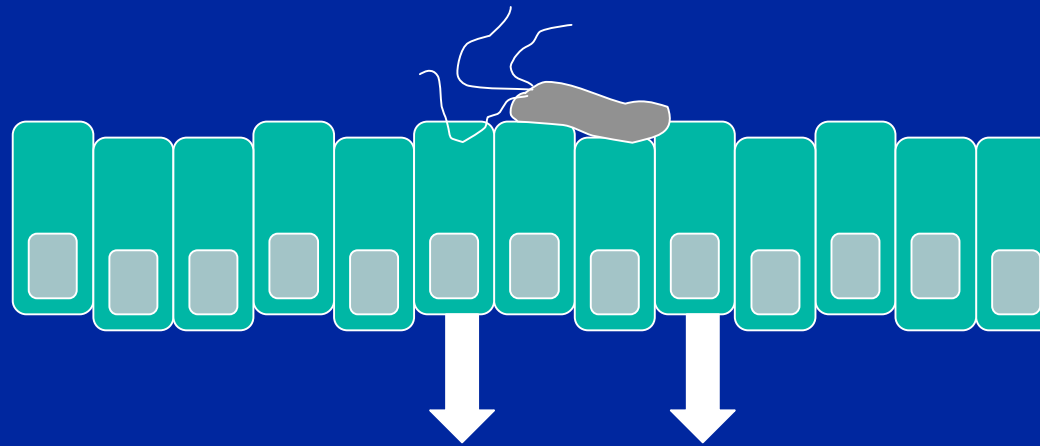
Gastric diseases can be cured and are preventable

# Possible mechanisms of action of *H. pylori* infection on gastric carcinogenesis

- Indirect action of *H. pylori* infection:  
*Numerous arguments*
- Direct action of *H. pylori* itself:  
*possible, but to be proven*
  - carcinogen production?
  - inhibition of defense mechanisms of the host?

# Inflammatory response.

Direct contact of *H. pylori* with epithelial cell



Chemokines :

IL-8

GRO- $\alpha$

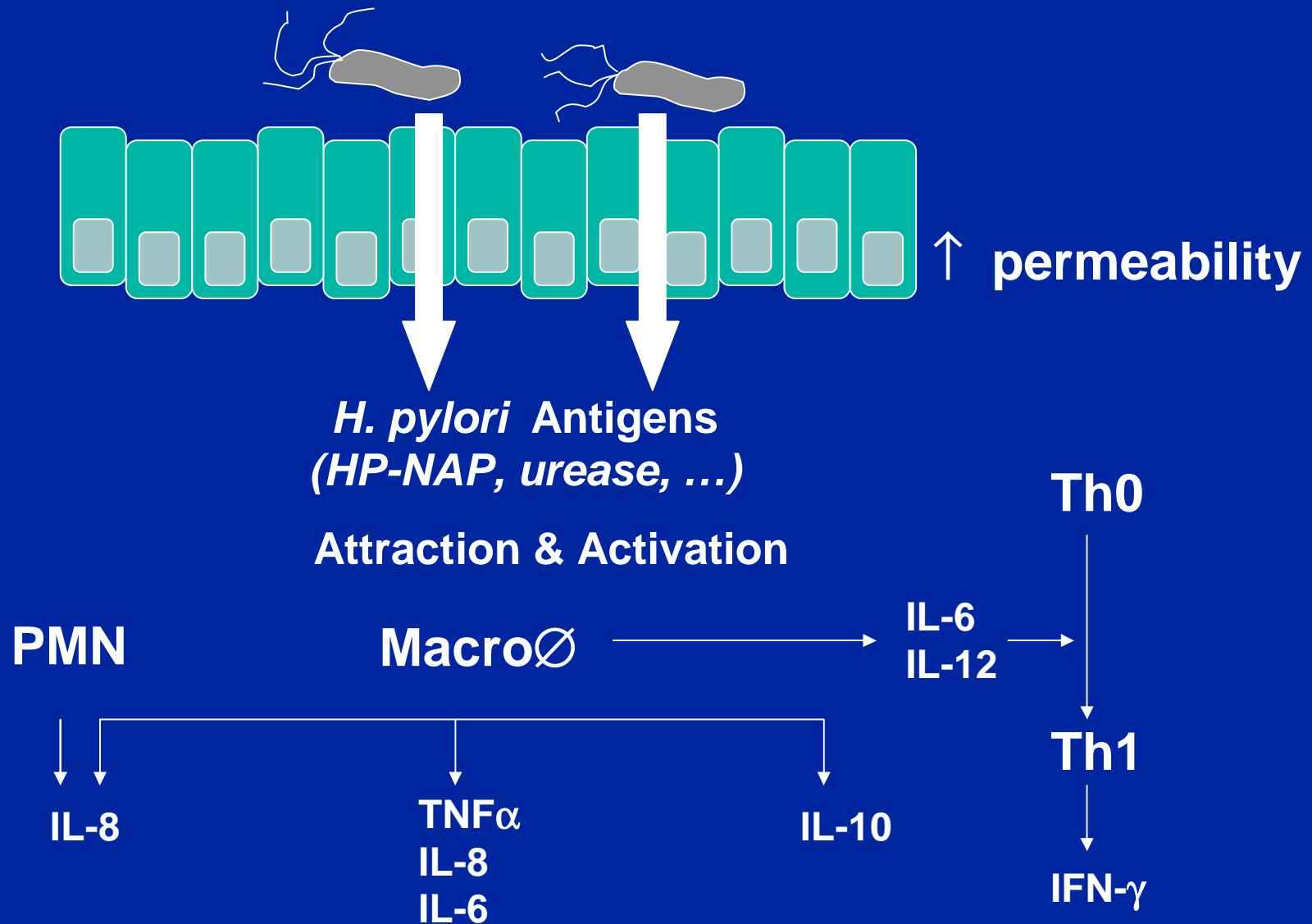
ENA-78

Attraction & Activation

PMN

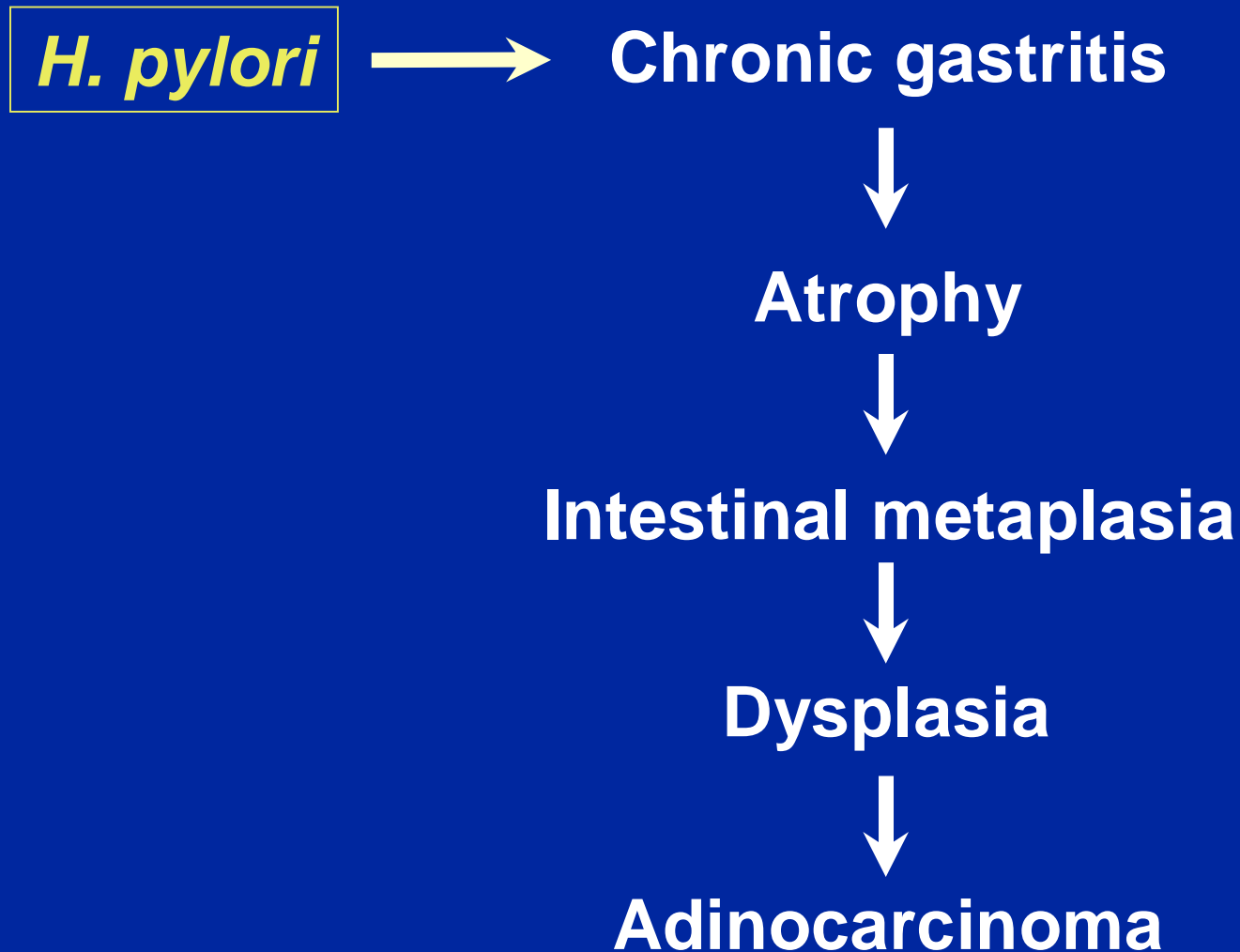
Macro $\emptyset$

# Inflammatory response. Innate response.

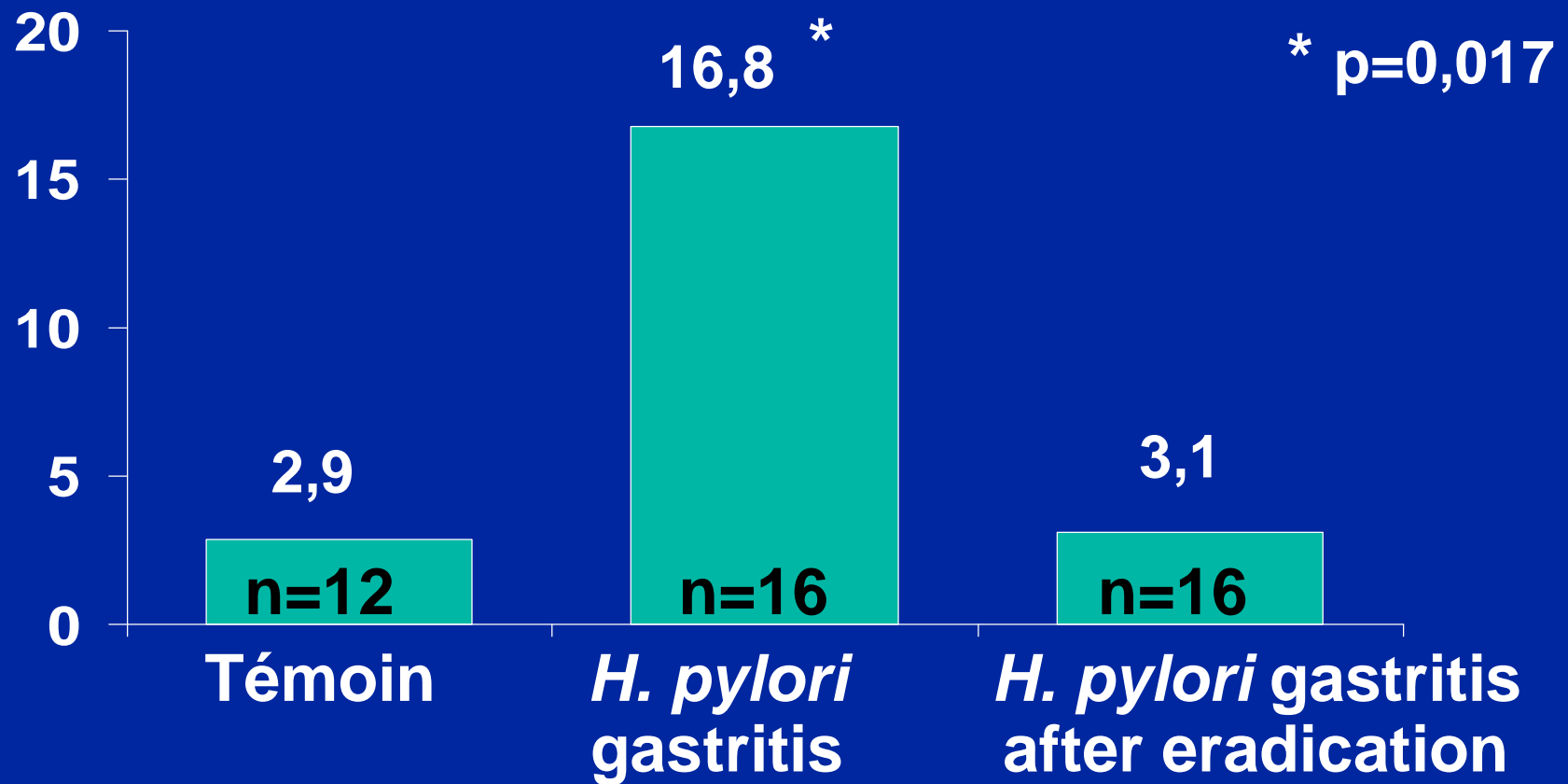


# Gastric cancer cascade

according to P. Correa



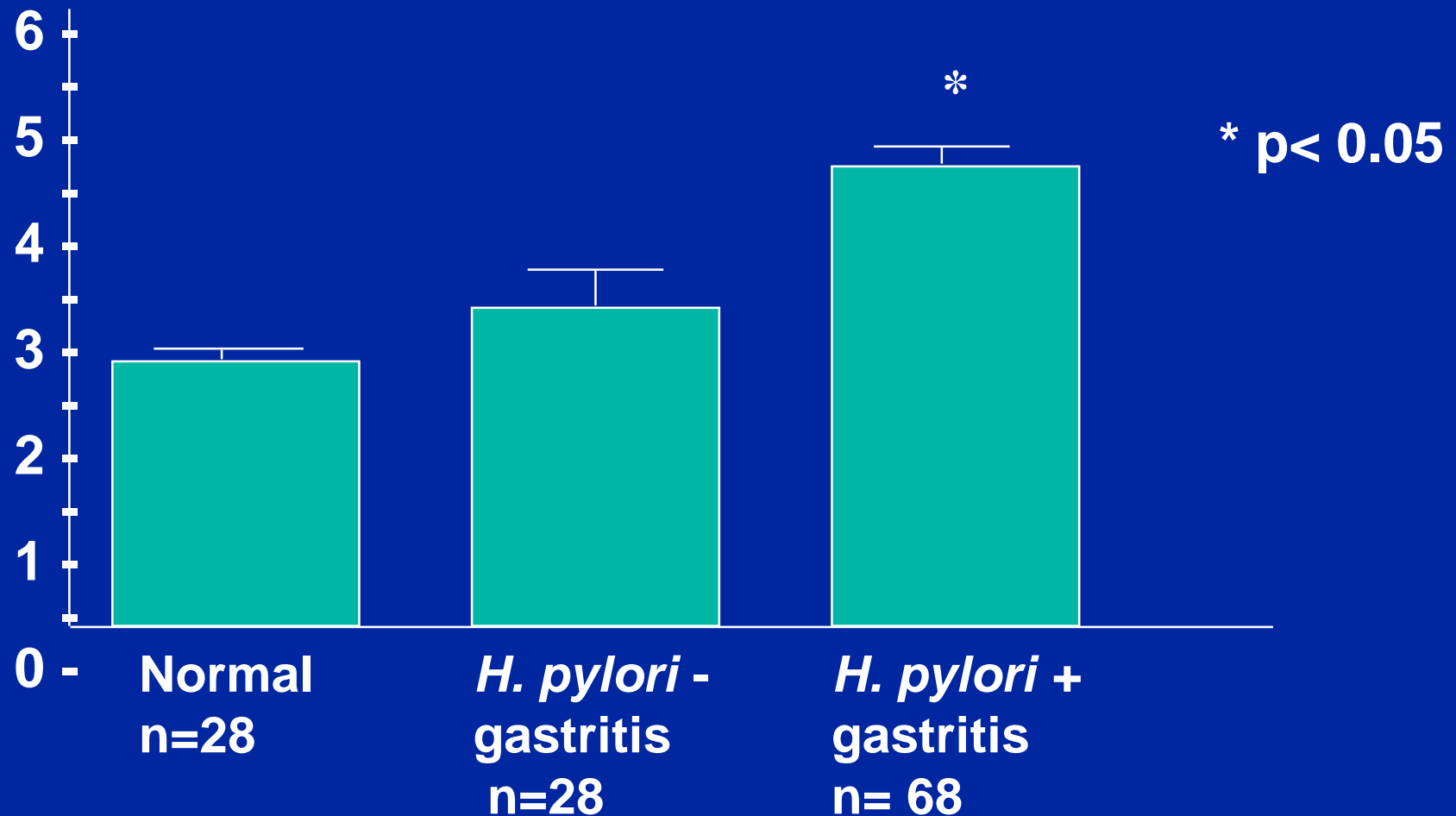
# Effect of *H. pylori* on gastric cell apoptosis (TUNEL Method)



*Moss et al., Gut 1996, 38: 498-501*

Comparison of the total labelling index percentage (LI%) for patients with normal mucosa, *H. pylori* - gastritis and *H. pylori* + gastritis.

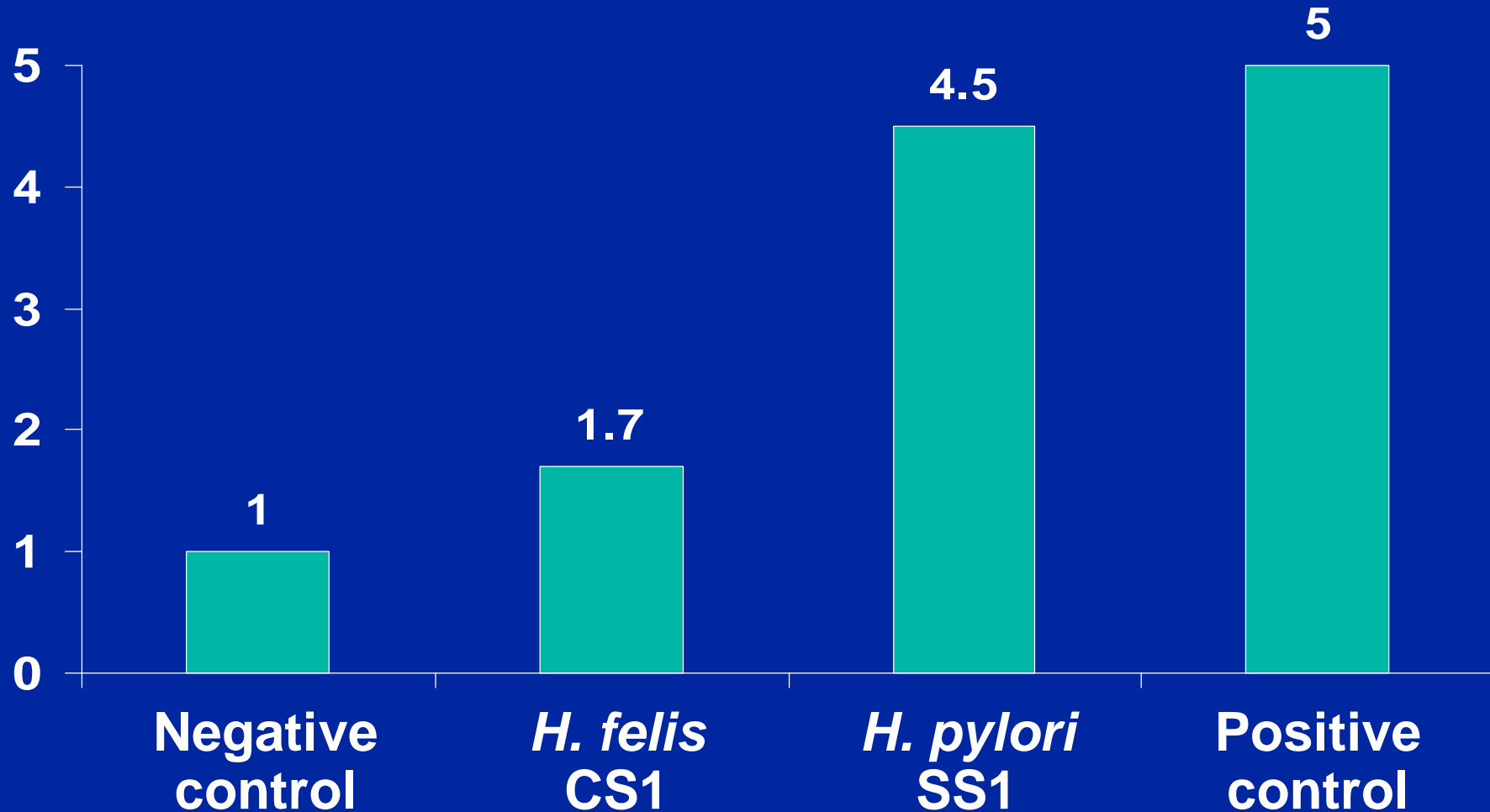
Total 4%



*Cahill et al, Eur J Gastroenterol Hepatol, 1994, 6, 1123-8*

# Impact of *H. pylori* on host cell mutagenesis

Model: Big blue transgenic mice infected for 6 months



*Touati et al., Gastroenterol Clin Biol 2002;26:A110*

# *H. pylori* impairs DNA mismatch repair in gastric epithelial cells

- inhibition of the gene concerned (RNAm of Mut S and Mut L)
- dose dependant decrease of the corresponding proteins
- caused by heat sensitive *H. pylori* products

➔ mutation accumulation during ADN replications

*Kim et al., Gastroenterology 2002;123:542-53*

# Factors which trigger the evolution from gastritis to gastric carcinoma

Environmental factors

Host factors



A diagram illustrating the factors that trigger the evolution from gastritis to gastric carcinoma. At the top, 'Environmental factors' and 'Host factors' are written in white text. Two yellow arrows point downwards from these terms towards a central white box with a black border containing the text 'Gastric carcinoma'. Below this box, a single yellow arrow points upwards towards the box, with the text 'Bacterial factors' written in white below it.

Gastric carcinoma

Bacterial factors

Are some strains more carcinogenic than others?

# Bacterial factors

## Genotypic variability of *H. pylori*

- **Microdiversity studies**

molecular typing methods all non epidemiologically related strains are virtually different

- **Macrodiversity studies**

DNA chips: 20% of genes are different  
high level of genetic rearrangements

# *H. pylori* virulence factors

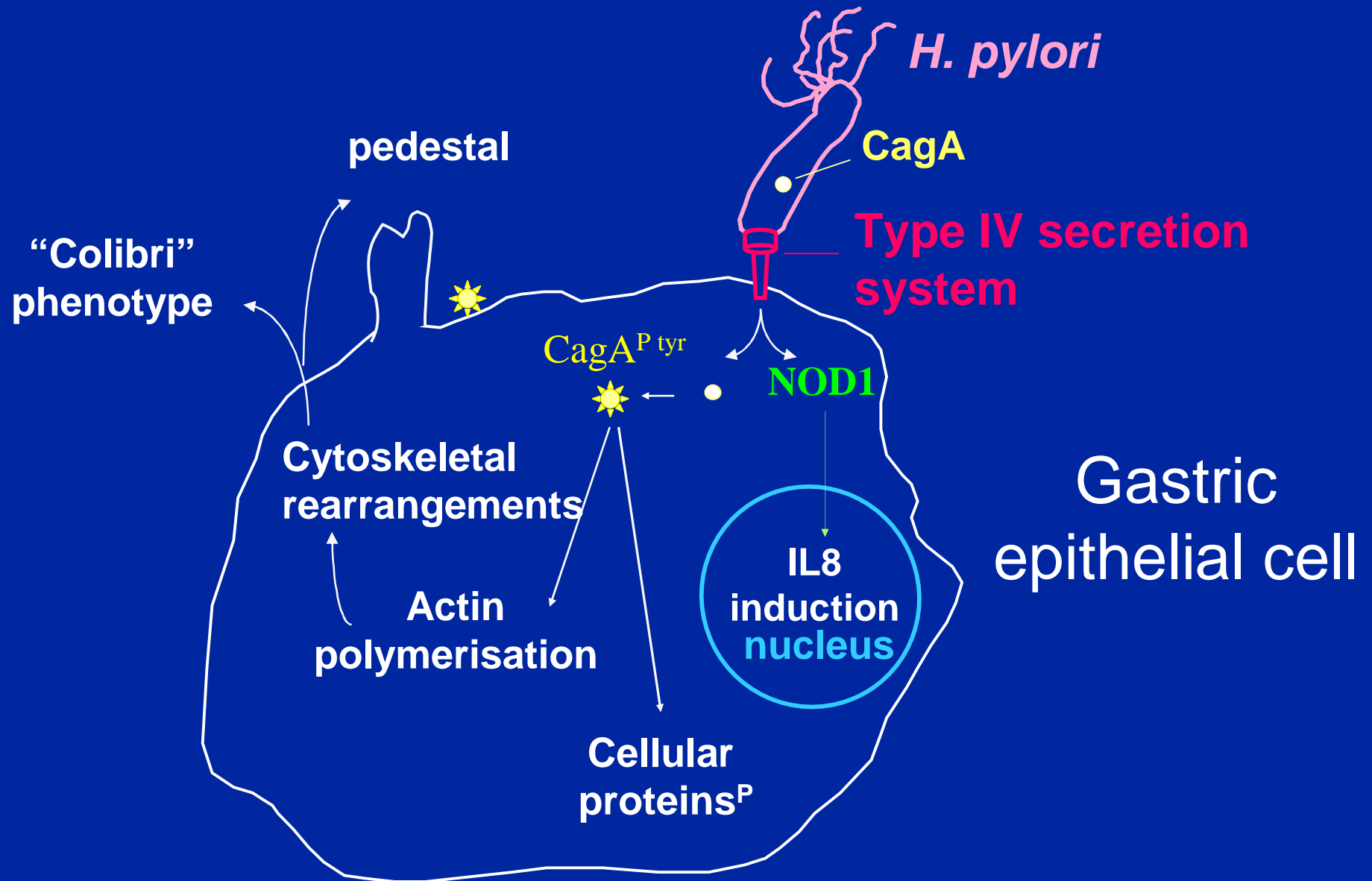
- Outer membrane proteins
  - adhesins : *babA2*, *sabA*
  - *hopZ*, *hopQ*
- Inflammation (IL-8 induction)
  - *cag* pathogenicity island
  - *oipA*
  - *iceA1*
- VacA vacuolating cytotoxin

# *H. pylori cagA*<sup>+</sup> is a risk factor of gastric cancer of intestinal or diffuse type

	Patients		Controls		OR
	<i>cagA</i> <sup>+</sup>	<i>cagA</i> <sup>-</sup>	<i>cagA</i> <sup>+</sup>	<i>cagA</i> <sup>-</sup>	
All gastric carcinomas	113 (95%)	6	79 (66%)	40	9.5 [3.6-26.8]
Intestinal type	76	3	54	26	11.7 [3.1-33.7]
Diffuse type	30	2	22	13	9.8 [1.8-39.7]

Queiroz et al., *Int J Cancer* 1998, 78: 135-9

# Role of cag PAI and CagA protein



*Covacci & Rappuoli, J Exp Med 2000;191:587-92*

# CagA protein in the gastric cell

- Tyrosine phosphorylation by Src family kinases on

EPIYA motifs

A

B

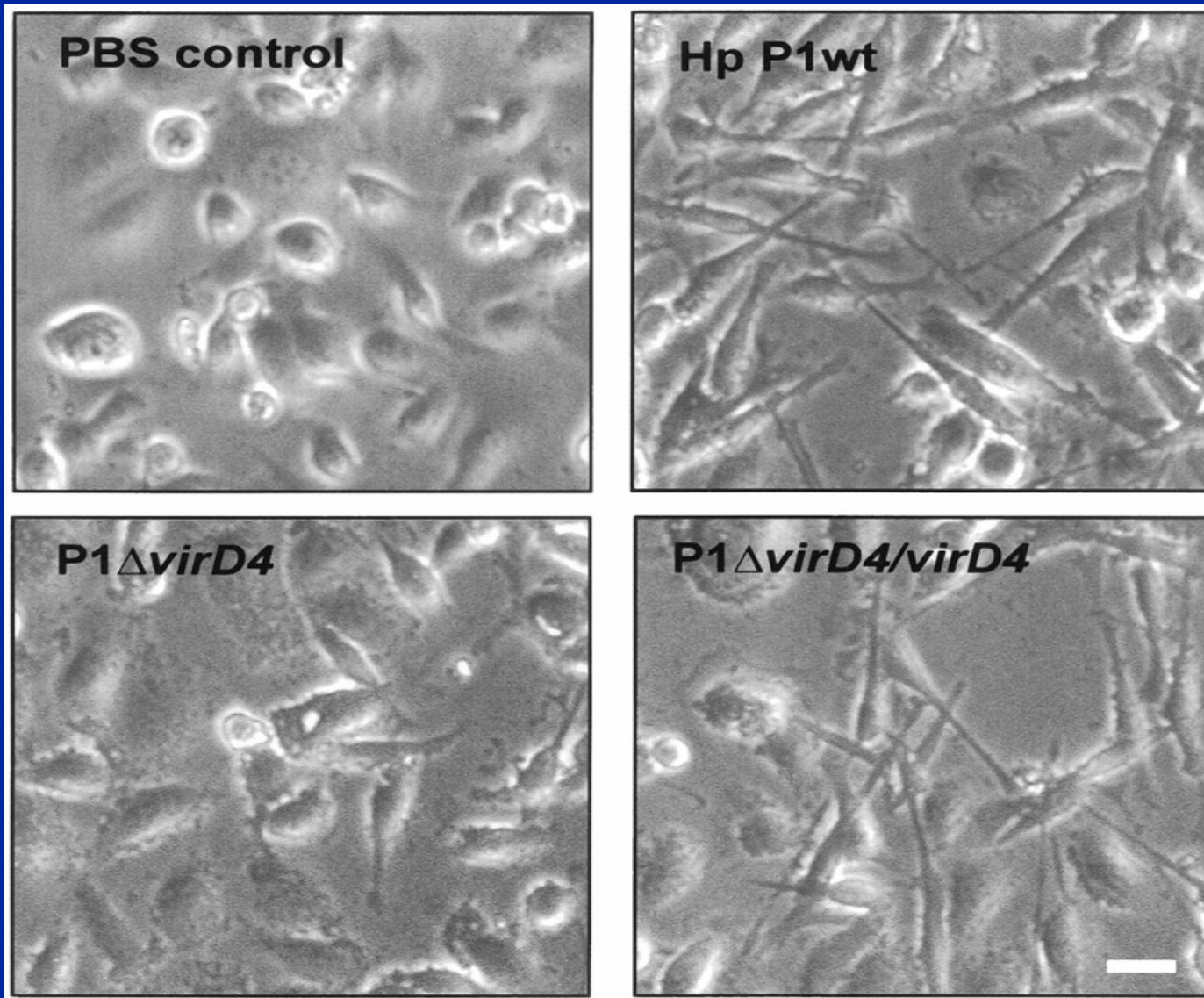
C Western Hp

D Eastern Hp

- Binding to the tyrosine phosphatase SHP-2  
CagA-SHP-2 participates in signal transduction & regulation of proliferation, cell motility via ESK MAP kinase activity  
stronger binding of Eastern CagA  
feed back on Src family kinases to avoid session toxicity

*Hakateyama & Higashi Cancer Sci 2005;96:835-43*

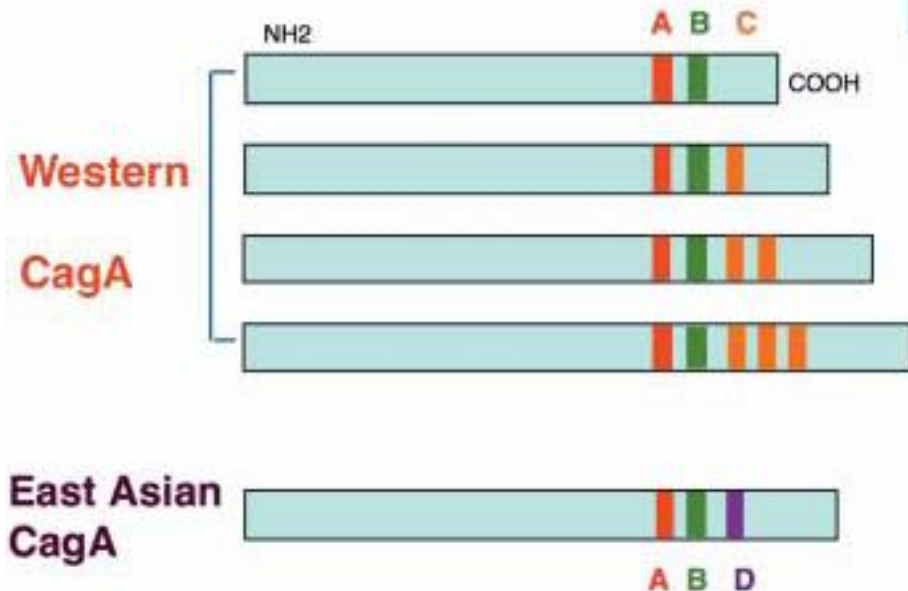
# Induction of the “humming bird” phenotype in AGS cells



*Selbach et al., Infect Immun 2002, 70: 665-71*

# Influence of EPIYA polymorphism on the pathophysiological activities of CagA

(b)



CagA type	Tyrosine phosphorylation	Induction of hummingbird phenotype	Oncogenicity
AB	±	±	±?
ABC	+	+	+?
ABCC	++	++	++?
ABCCC	+++	+++	+++?
ABD	+	++++	++++?

# **CagA protein in the gastric cell (2)**

**Phosphorylation independant activity:**

**association with tight-junction proteins (ZO-1)**  
—→ **alteration of the apical-junctional complex**  
—→ **disruption of the epithelial barrier**

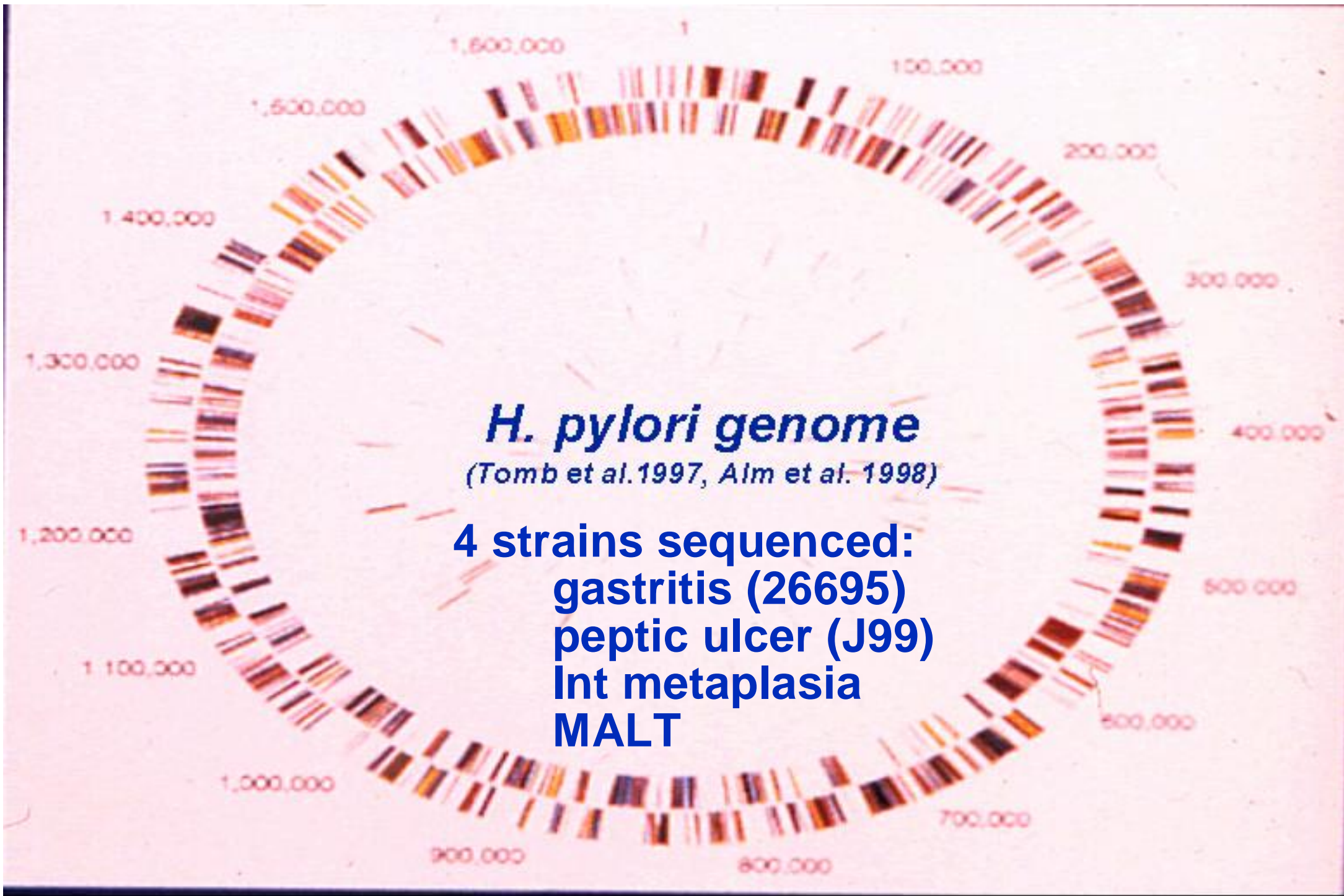
*Hakateyama & Higashi Cancer Sci 2005;96:835-43*

# Association of *vacA* s1m1 genotype with gastric carcinoma

	Gastric carcinoma	Controls	
<i>vacA</i> s1m1	+	24	12
	-	10	23
	34 (70.6%)	35 (34.3%)	p=0.005

but all *vacA* s1m1 were also CagA+

*Miehlke et al., Int J Cancer 2000, 87: 322-7*



## ***H. pylori* genome**

*(Tomb et al. 1997, Alm et al. 1998)*

**4 strains sequenced:  
gastritis (26695)  
peptic ulcer (J99)  
Int metaplasia  
MALT**

# Host factors

Some interleukin 1  $\beta$  genotypes

(IL1  $\beta$  - 31T & IL1 RN 2/2)

→ ↑ IL1  $\beta$  (*powerful acid inhibitor*)

→ ↑ risk of chronic hypochlorhydria  
when *H. pylori* present:

↑ risk of gastric carcinoma

*El-Omar et al., Nature 2000, 404: 398-402*

## Combination of the host risk with the risk due to *H. pylori*

	OR	95% CI
Host genetic polymorphisms (IL-1B-511*T/IL-RN*2 homozygotes)	3.3	1.3 - 8.2
CagA+ <i>H. pylori</i>	15	7.8 - 29
Both together	25	8.2 - 77
VacAs1 <i>H. pylori</i>	17	7.8 - 38
Both together	87	11 - 679

*Figueiredo et al., J Ntl Cancer Inst. 2002,94:1680-7*

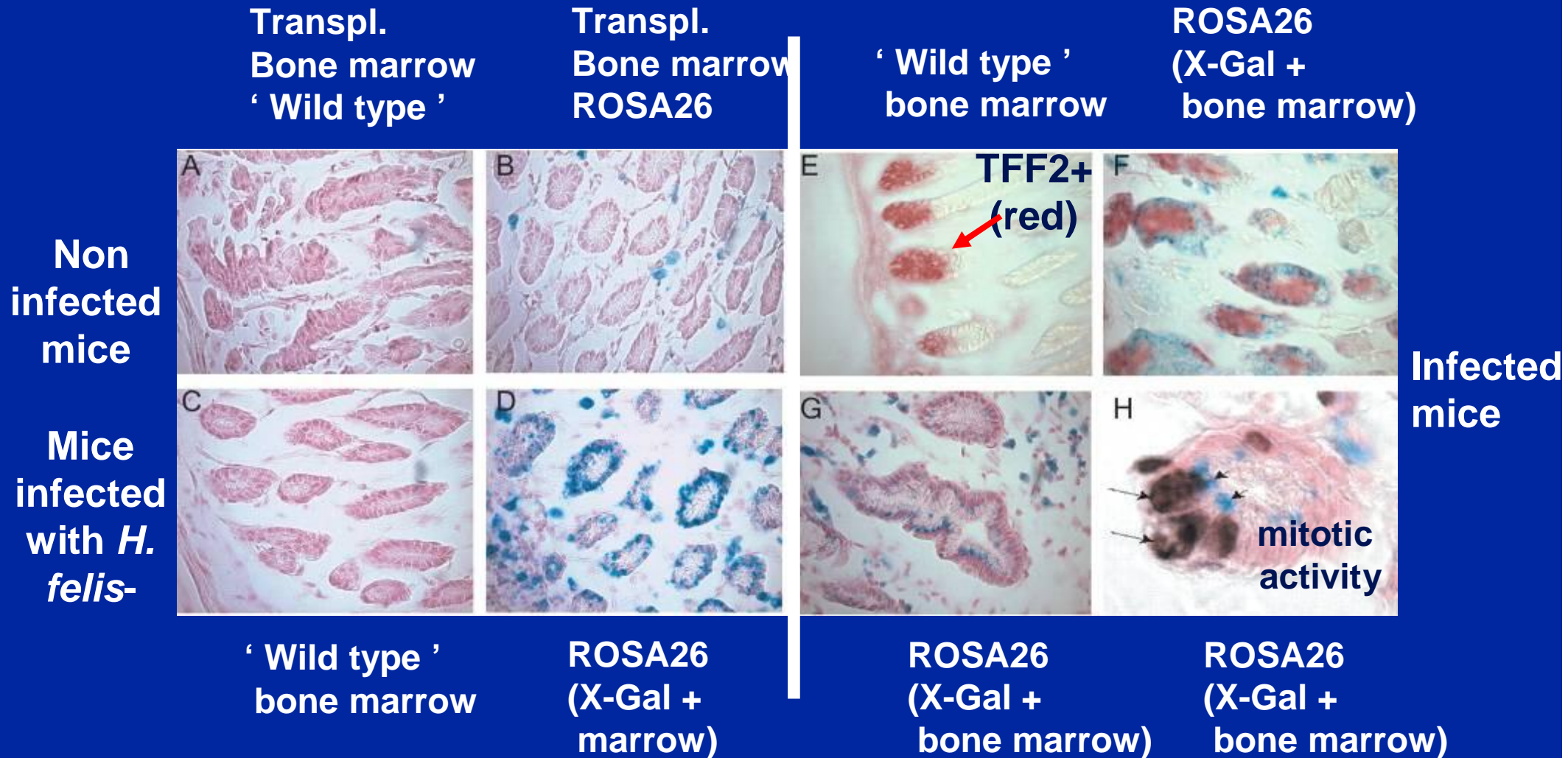
# **New hypothesis on pathogenesis of gastric carcinoma associated with Helicobacter infection:**

**Gastric cancer originates from bone marrow – derived cells**

- Recruitment of bone marrow mesenchymatous cells**
- Inflammatory environment leads to cancer cells**

*Houghton et al, Science 2004;306:1568*

# Microscopic observations in the mouse stomach



# Diagnostic of *H. pylori*

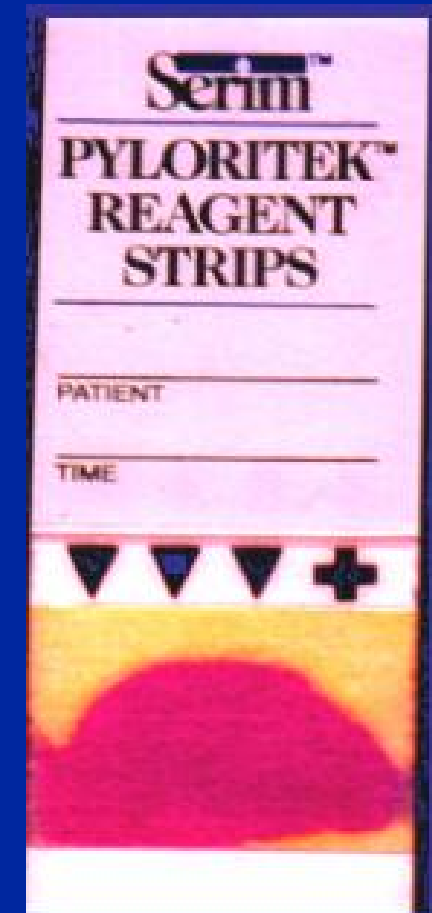
# Rapid urease test

## Principle

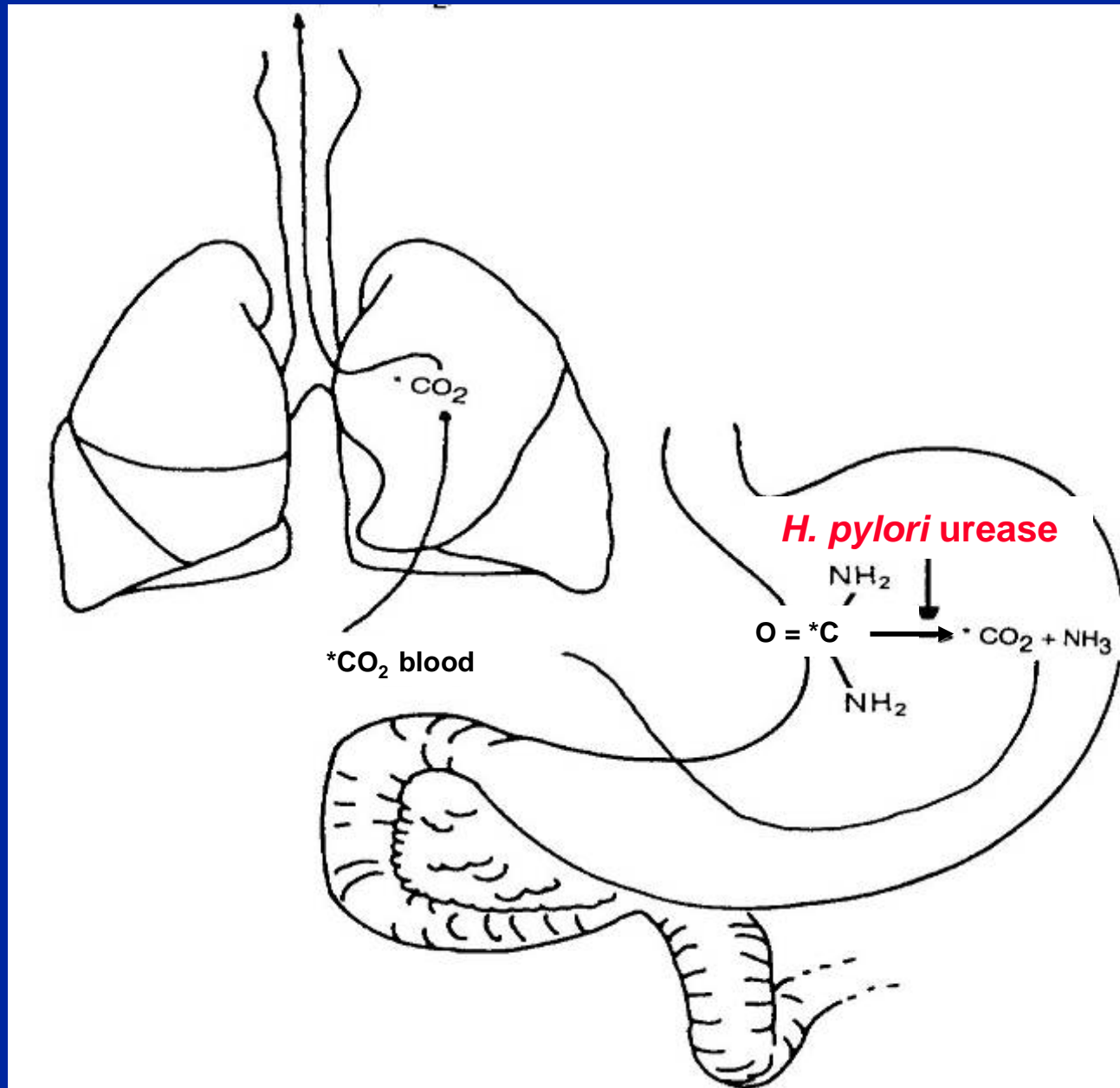
### Urease of *H. pylori*

- Urea → Ammonia + Carbon dioxide
- Ammonia raises the pH
- A pH indicator changes colour from **yellow** to **red**

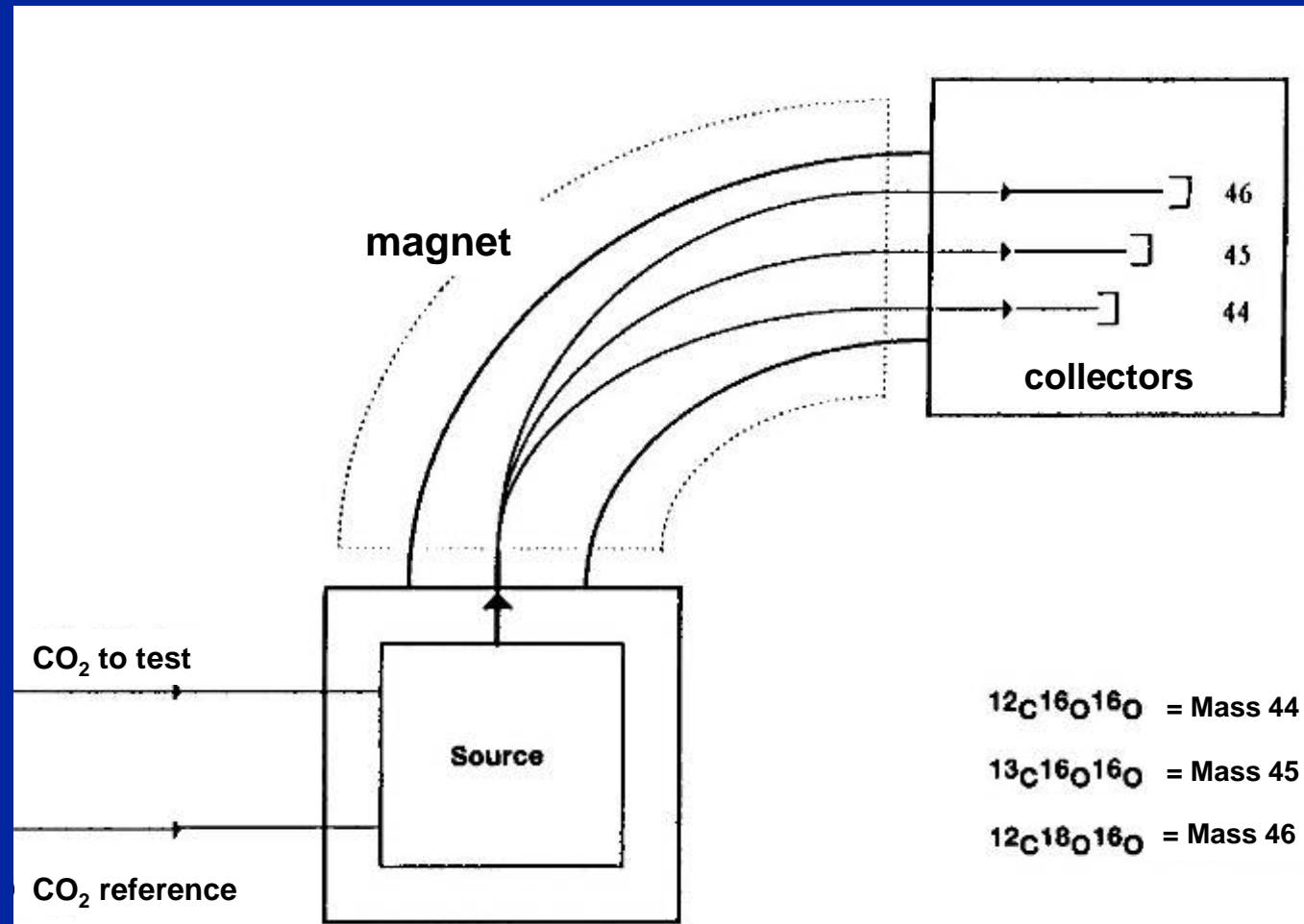
# Examples of Rapid Urease Tests



# Principle of the Urea Breath Test



# Principle of the measurement of $^{13}\text{CO}_2$ using mass spectrometry



# Tests in stools

- Detection of *H. pylori* antigens
- Detection of *H. pylori* DNA

# Stool Antigen Tests

## *Principle*

- Bacteria from the stomach are eliminated in stools
- *H. pylori* does not survive in stools but antigens and DNA can be detected
- Antibodies directed against *H. pylori* antigens are used to perform different types of tests

# Stool Antigen Tests

*Different tests available*

## 1. ELISAs

using polyclonal antibodies

using monoclonal antibodies

- Rapid immunoenzymatic tests (« doctor tests »)

using monoclonal antibodies

# *H. pylori* antigen detection in stools (ex: Premier Platinum HpSA<sup>®</sup> Meridian)

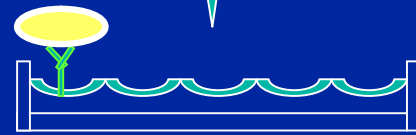
**ELISA**



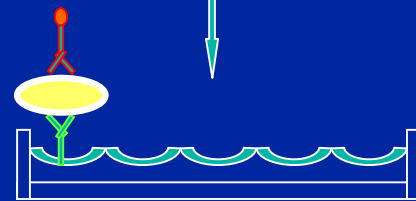
Ab coated



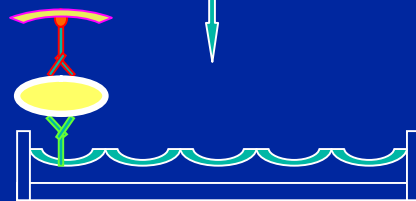
+ patients stools



+ conjugate



+ enzyme substrate



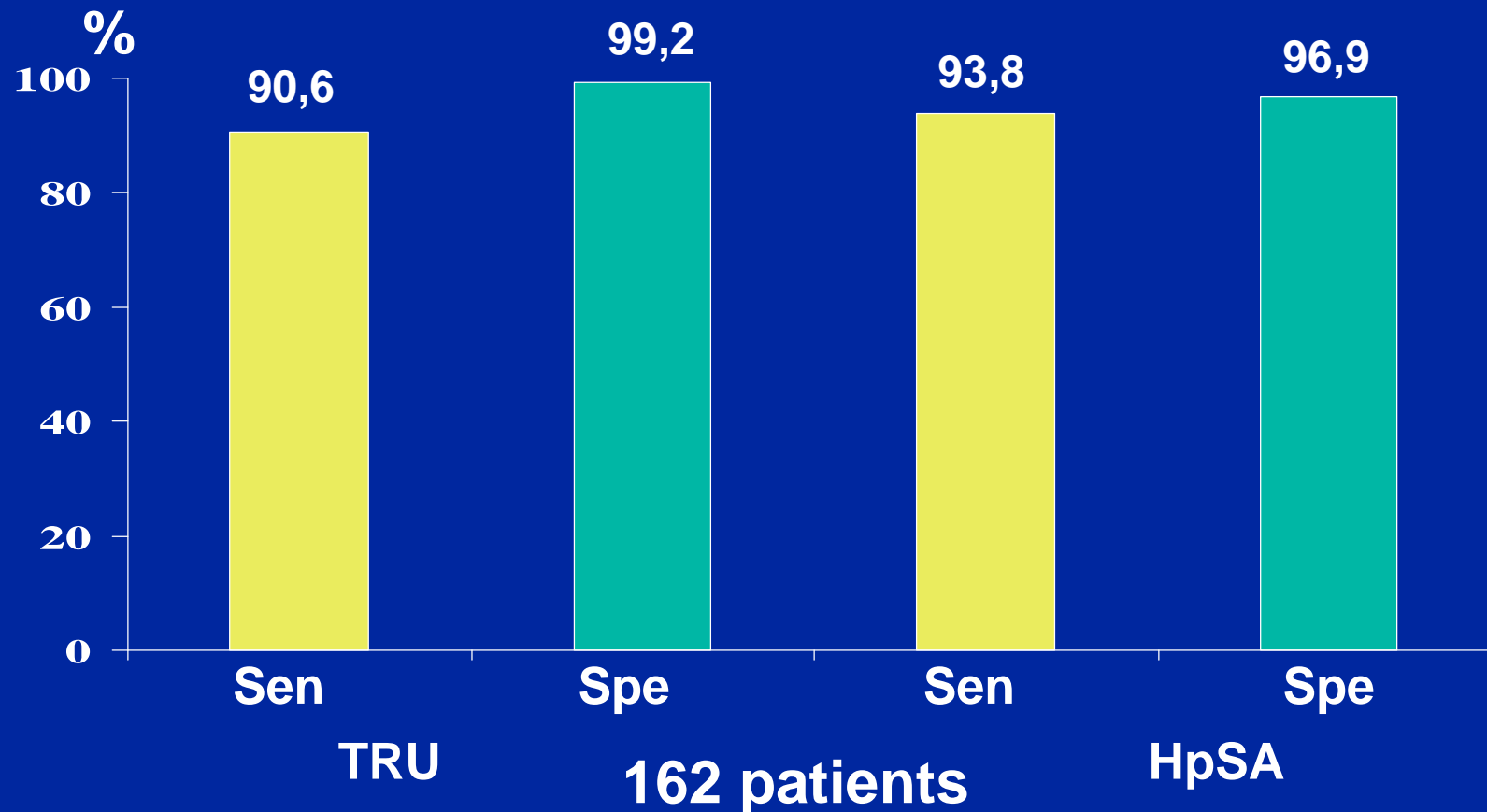
OD measured

# New tests based on monoclonal antibodies

- **ELISA :**  
HpStAR® (Dako) ex FemtoLab® (Connex)
- **Rapid test (doctor test) :**  
ImmunoCard STAT HpSA® (Meridian)



# Comparison between UBT and HpSA for post treatment follow up in adults



*Vaira et al. Am J Gastroenterol 2000;95:925-9*

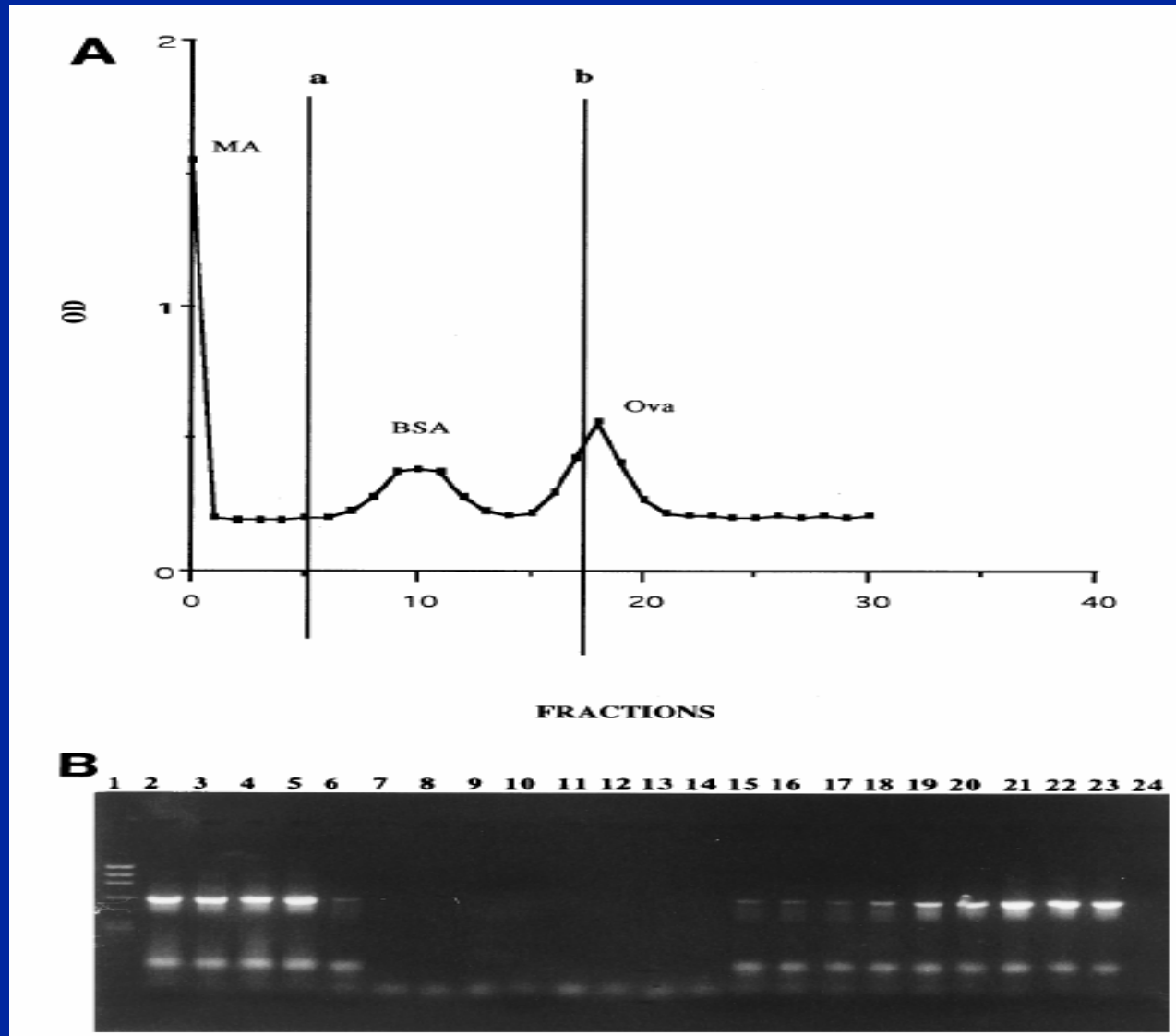
# PCR in stools

- Numerous attempts during the '90s

- Major problems:

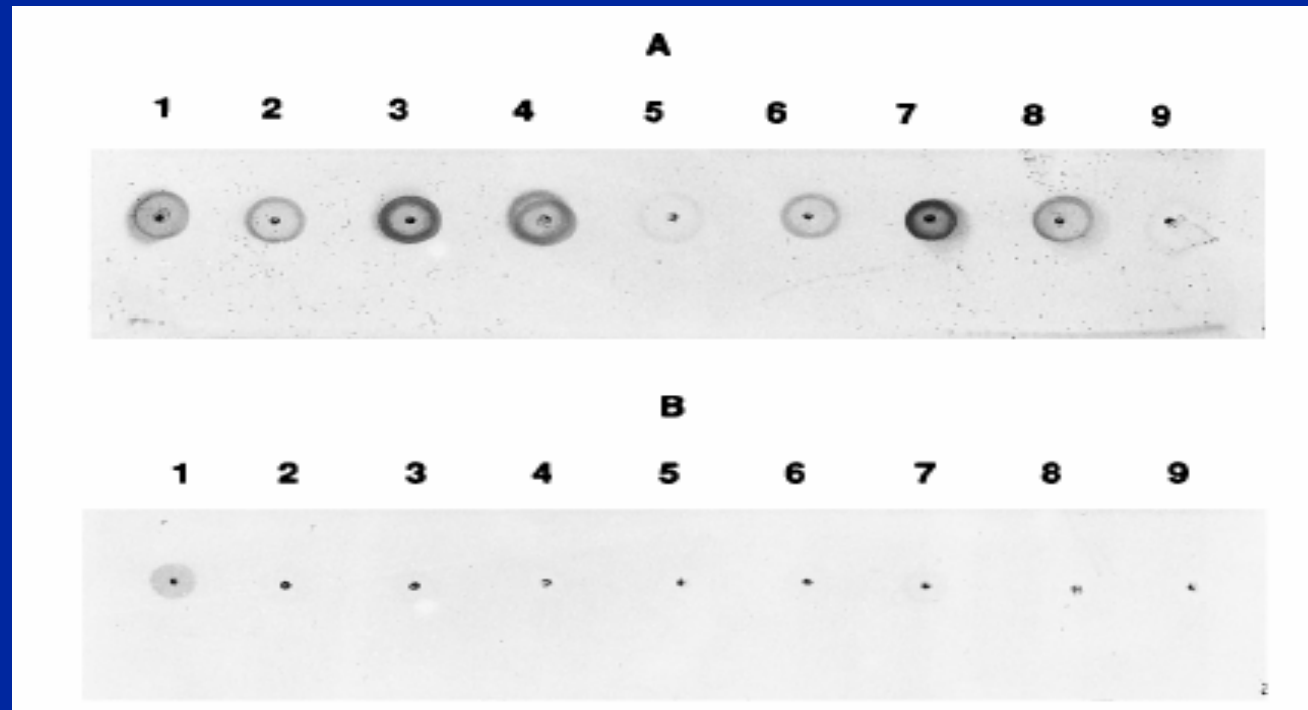
  - limited amount of *H. pylori* DNA
  - presence of PCR inhibitors

# Separation of stool fractions containing inhibitors with corresponding PCRs



*Monteiro et al.*  
*JCM*  
*1997;35:995-8*

# Identification of PCR inhibitors by thin-layer chromatography

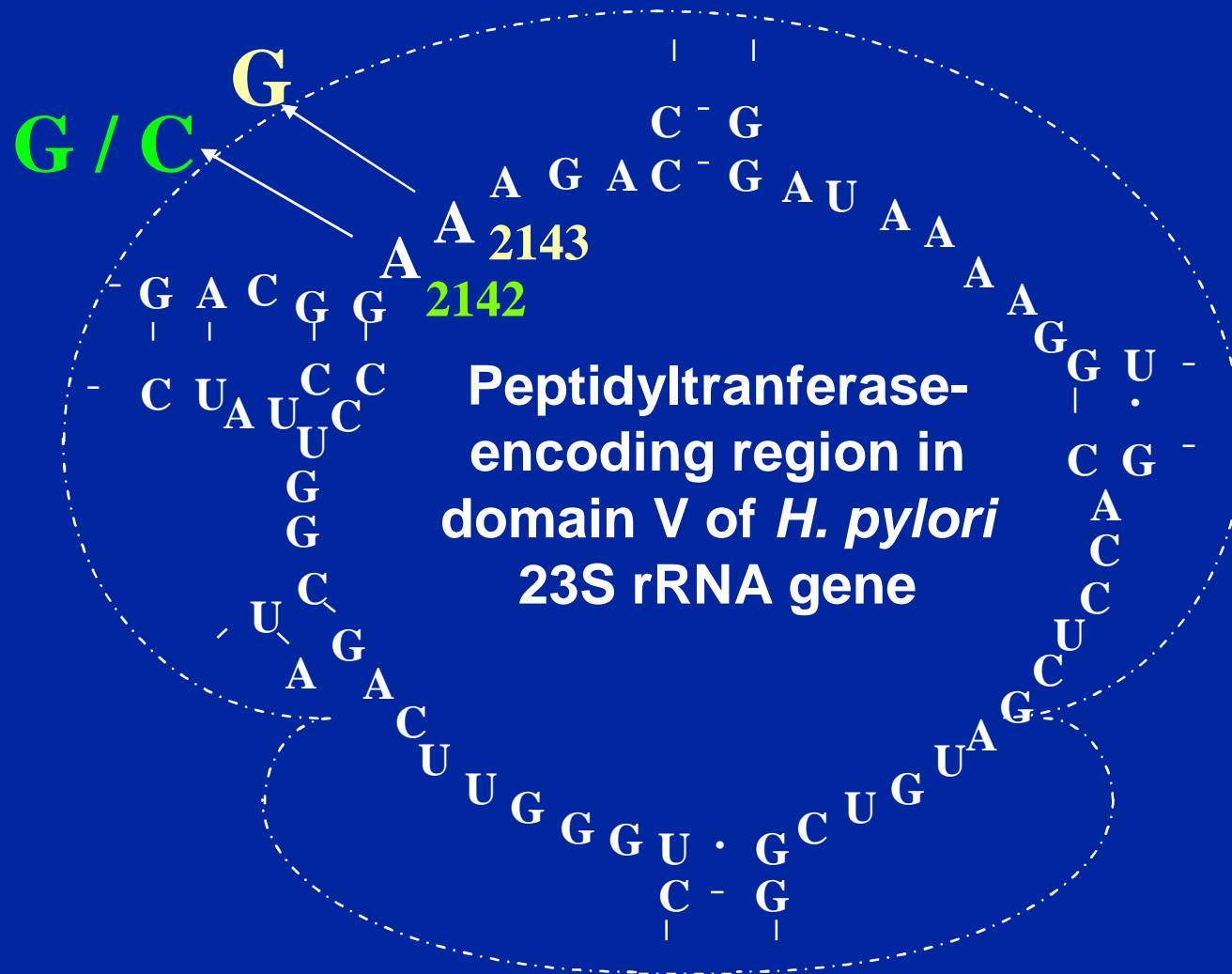


A: positive orcinol reaction → carbohydrates

B: negative ninhydrin reaction → no amino acids

*Monteiro et al. JCM 1997;35:995-8*

# Mechanism of *H. pylori* resistance to macrolides



Point mutations



Change in ribosome structure



Lack of binding of the macrolide

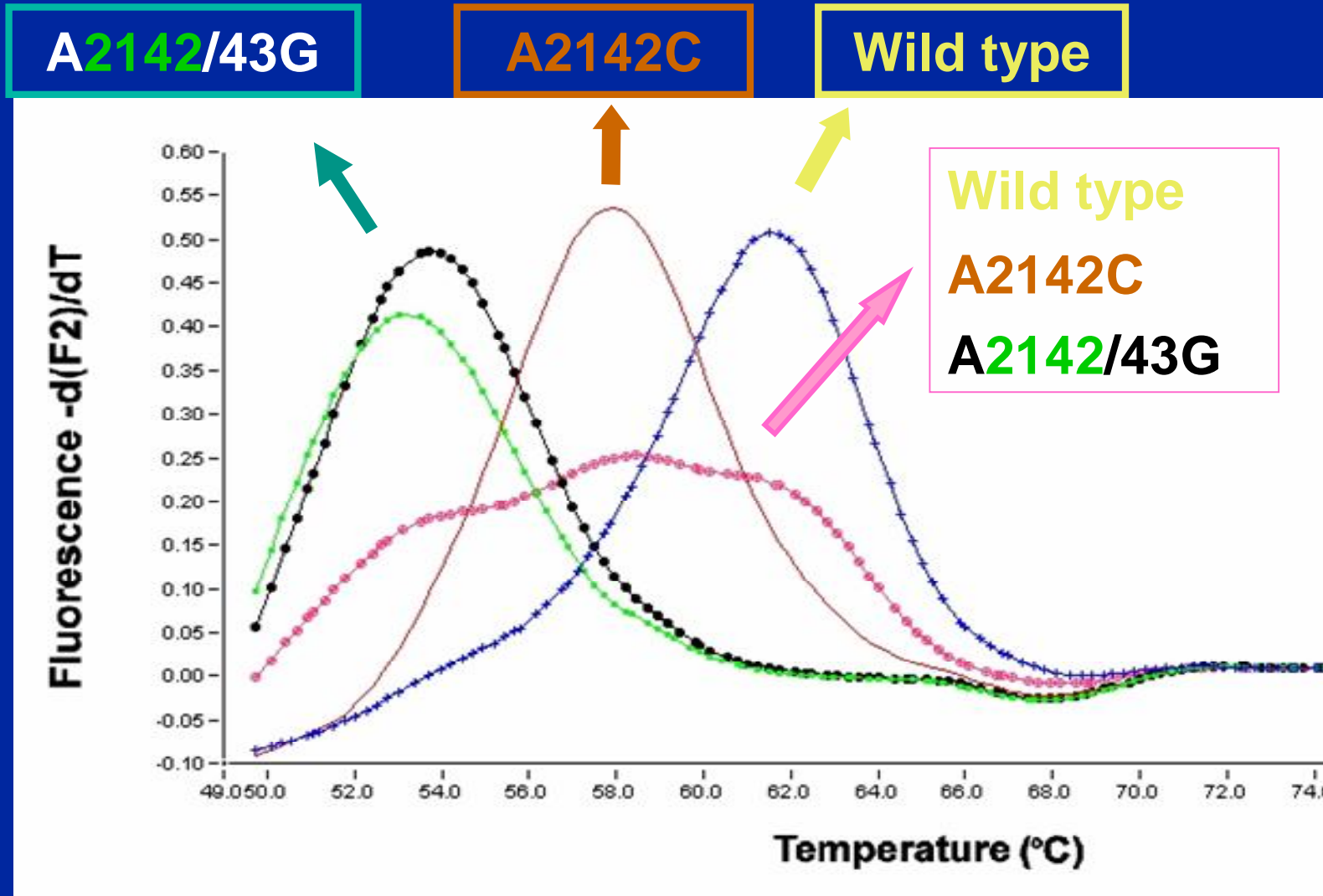


Macrolide resistance

*Taylor et al., 1997*



# Detection of the different point mutations on control strains by melting curve analysis



# Detection of *H. pylori* with a real-time PCR FRET assay

**Biopsy reception**

1 hour



Biopsy disruption, bacterial lysis & DNA extraction

**DNA**

45 minutes



PCR (LightCycler®)

**Identification & point mutation detection**

**Protocol duration: 2 hours**

Def

t In



# Developments of antibody tests

- **Detection of specific antibodies using recombinant antigen**
- **Use of whole blood “doctor tests”**
- **Use of immunoblot**
- **Detection of salivary IgG antibodies**
- **Detection of urinary IgG antibodies**

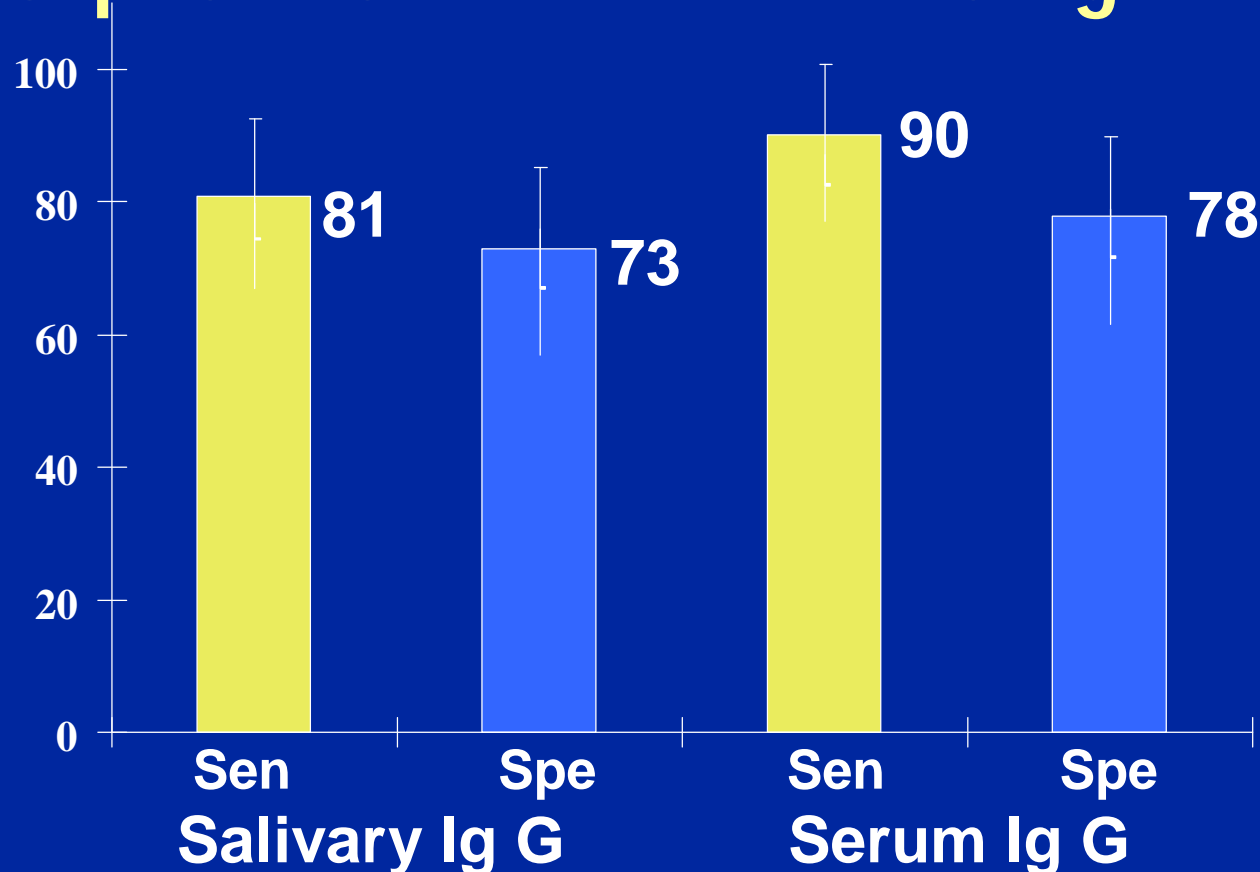


10/01/01

# Diagnosis of *H. pylori* infection by salivary Ig G

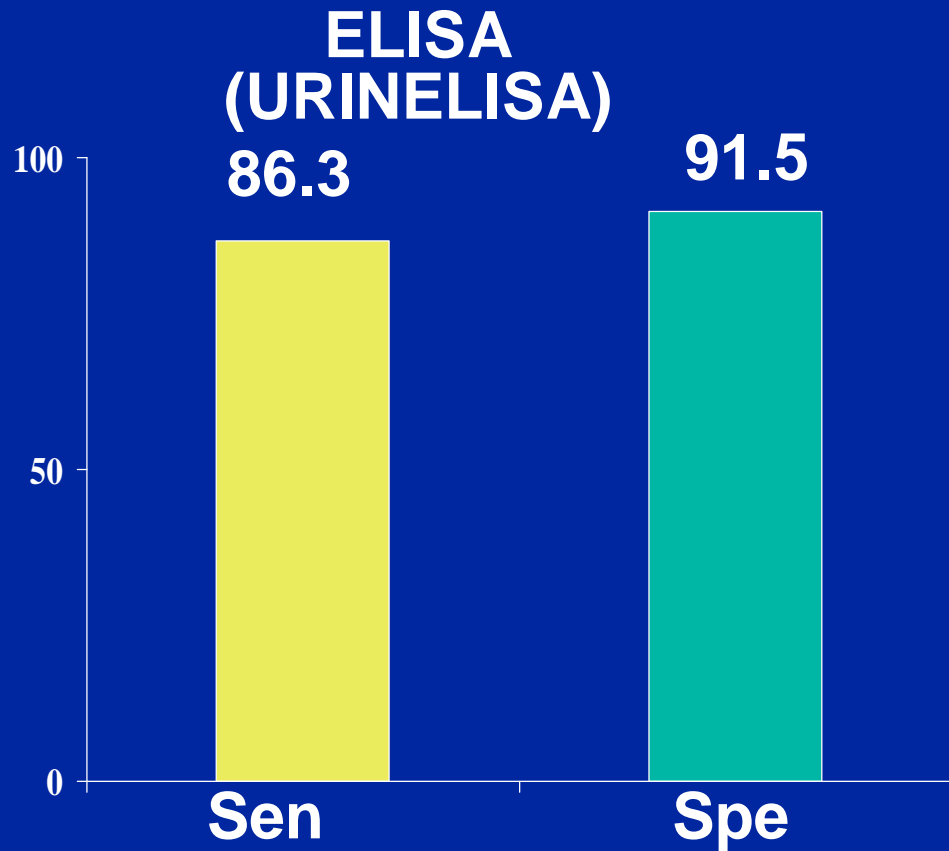
Multicentric study in adults.

213 patients. Helori-Test Ig G kit

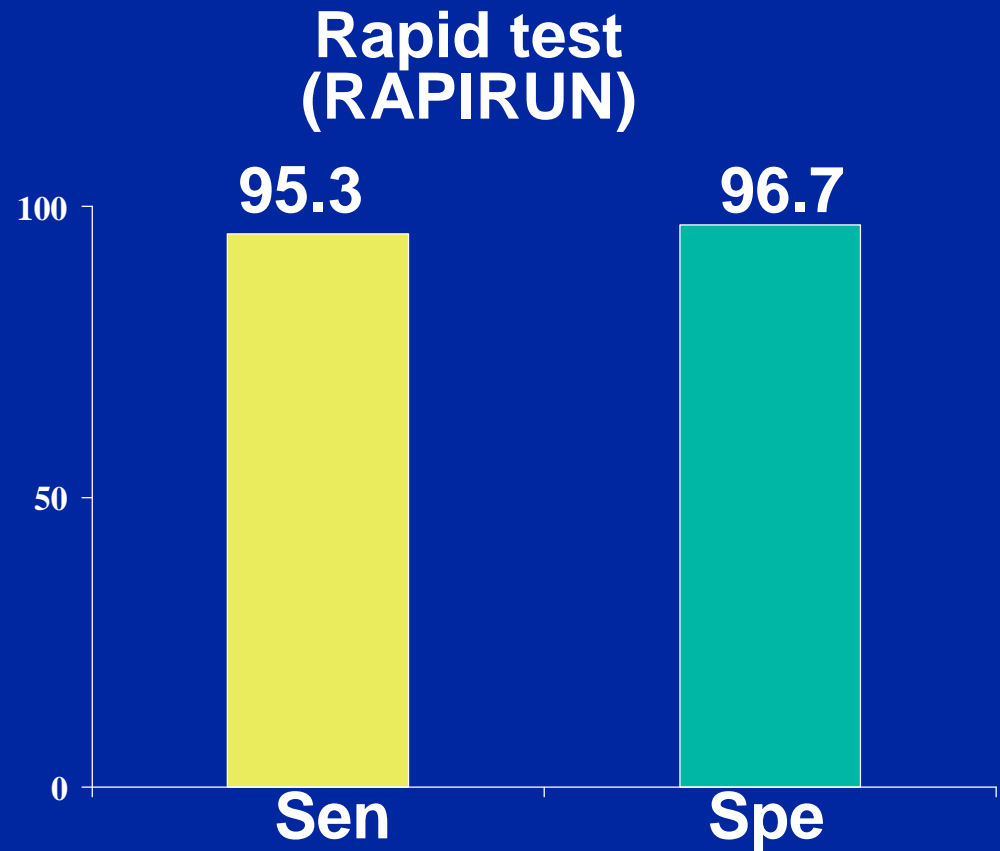


*Luzza et al EJGH 2000;12:117-20*

# Detection of anti *H. pylori* antibodies in urine



Reference : UBT  
*Miwa et al*  
*Am J Gastroenterol*  
*1999;94:3460-3*



Reference : UBT  
*Graham & Reddy*  
*APT 2001;15:699-702*

**Maastricht 3-2005**

**Management of *H. pylori* infection**

# Management of dyspeptic patients in the community

Dyspeptic patient (1st visit to a general practitioner)

<45 years without alarm symptoms  
Examination of history

>45 years in any case or with  
alarm symptoms if younger

Test for *Hp*-Current laboratory tests( urea  
breath test or stool test) recommended

if *Helicobacter pylori* +

*Helicobacter pylori* eradication  
Treatment given by GP

Referral to the gastroenterologist

(This falls into the category advisable  
with equivocal evidence)

*Malfertheiner et al., APT 2002;16:167-180*

# Disadvantages of serological tests

- requirements for validation especially high (cut-off values dependent on population studied, age group, prevalence...)
- differentiation between acute and past infection difficult
- lower sensitivity and specificity in children than in adults
- no uniform standards regarding antigens to be used and test formats
- partly only inadequately validated tests available

# Advantages of serological tests

- minimally invasive, “global” test
- detection of *H. pylori* also possible in cases with low pathogen density and/or bacterial overgrowth
- cost-effective and readily available
- suitable for testing large samples
- results are not affected by antibiotic or antisecretory therapy
- allows for indirect determination of *H. pylori*-specific virulence factors

# Diagnosis in patients receiving antisecretory drugs

- both invasive tests and stool or breath tests will be negatively affected
- *H. pylori* specific IgG will not change significantly under these treatments

# Sensitivity of UBT and stool antigen test before and after administration of proton pump inhibitors

<b>Test</b>	<b>Sensitivity (%)</b>	
	<b>before</b>	<b>after</b>
<b>Urea breath test</b>	<b>100</b>	<b>73-85</b>
<b>Stool antigen test</b>	<b>98</b>	<b>83</b>

*Gatta et al., Am, J. Gastroenterol. 2004*

# Diagnosis in patients with bleeding ulcers

- limited possibilities to take biopsies (emergency endoscopy, anticoagulant therapy)
- rather low sensitivity for the **RUT** (40-75%) in the majority of studies
- **histology and culture** also show a lower sensitivity than in patients without bleeding
- false negative results must be anticipated for the **UBT** (PPI pre-treatment)
- very low specificity (30%) of **polyclonal stool antigen tests** (cross reactivity with blood products)
- results of serology are neither affected by the presence of bleeding nor by pre-treatment with PPI

# Atrophic gastritis and serology

- Most cases with *H. pylori* detection exclusively based on serology were actually active infections!
- 16 patients with atrophic gastritis (histology and UBT negative, serology positive)

8 patients treatment group

8 patients control group

in 6 out of 7 significant  
decrease of titers

in 1 out of 8 significant  
decrease of titers

*Kokkola et al., J Clin Microbiol 1998*

# Sensitivity of diagnostic tests in 40 *H. pylori*-positive MALT-Lymphoma patients

Test	No pos.	% pos.
Histology	39	97,5
Culture	21	52,5
PCR	20	50,0
Serology	38	95,0

*Lehours et al., Am J Gastroenterol 2003*

# Diagnosis of *H. pylori* infection in the presence of gastric cancer

- diagnosis of infection in the presence of gastric cancer is **without any therapeutic consequence**, but is nevertheless essential for describing the association
- **seroprevalence is significantly higher** than the prevalence of direct detection tests (low pathogen density, uneven distribution, bacterial overgrowth)
- **serology (incl. *cagA*) and immunoblots** provide for the most reliable reflection of the association between *H. pylori* and gastric carcinoma

# Importance of bacterial pathogenic factors

Data exist on the association of:

- CagA with peptic ulcer disease and gastric adenocarcinoma (Queiroz et al 2002, Nomura et al 2002)
- *vacAs1m1* with peptic ulcer disease (Atherton et al 1997, Kidd et al 1999) and gastric adenocarcinoma (Miehlke et al 2000)
- BabA2 with peptic ulcer disease and gastric adenocarcinoma (Gerhard et al 1999)
- OipA with peptic ulcer disease (Yamaoka et al 2002)

# Importance of host genetic polymorphisms

## Concerns host cytokines

- Positive data

**El-Omar et al 2000, Figueiredo et al 2002, El-Omar et al 2003, Rad et al 2004, Chen A, et al 2004, Hartland et al 2004, Wu et al 2004**

- Negative data

**Garcia-Gonzales et al 2005**

## **But... .**

- **Relatively few supportive data so far, based on individual randomised controlled trials.**
- **Tests too expensive to be used on a large scale and questionable cost-effectiveness.**
- **Therefore, more data is needed before positive recommendation can be given.**

# Maastricht 2-2000 Consensus Report

## *"The treatment package"*

- **First line therapy**

PPI/RBC-Clarithromycin-Amoxicillin  
(bd) (500 mg bd) (1 g bd)

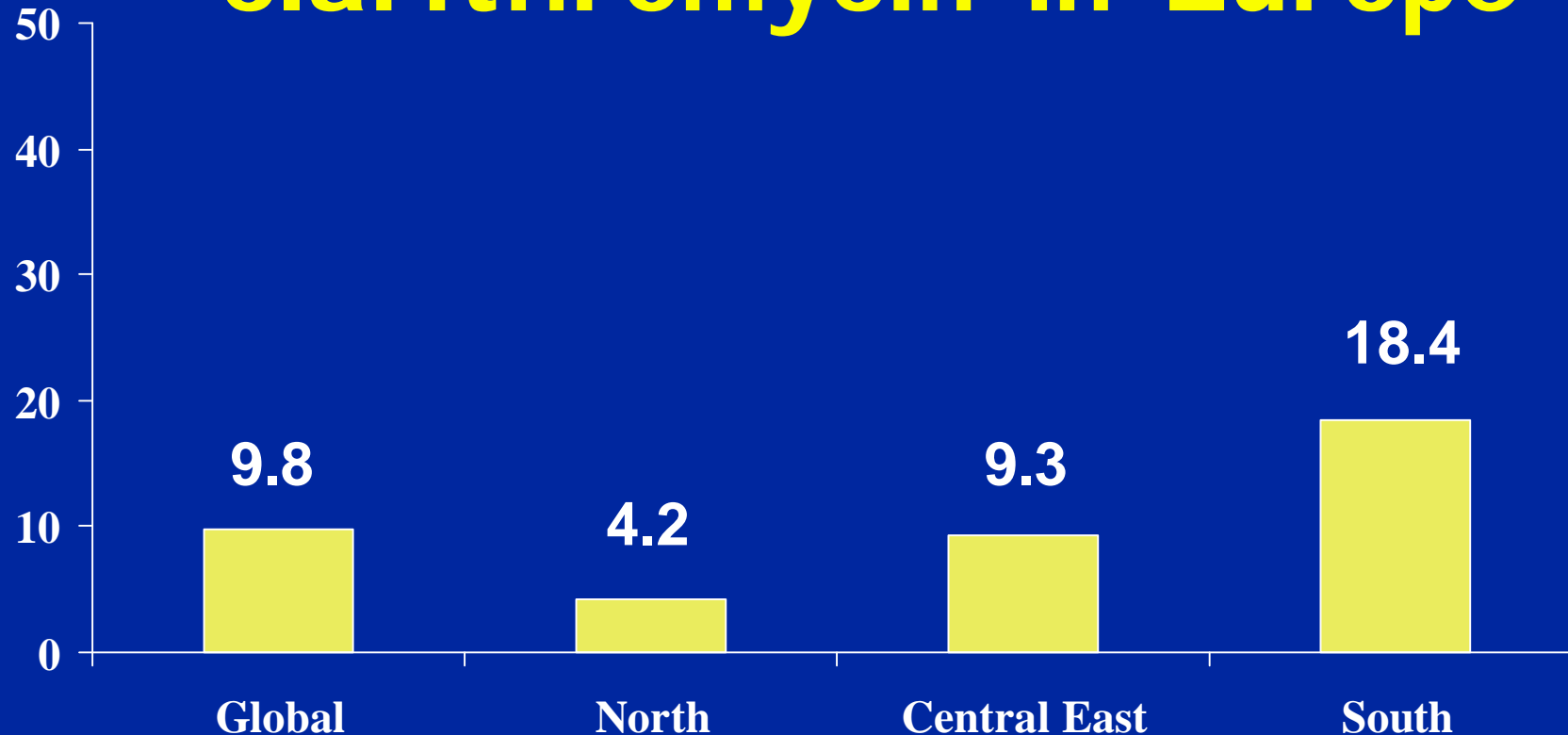
- **Second line therapy**

PPI-Bismuth-Tetracycline-Metronidazole  
(bd) (120 mg qd) (500 mg qd) (500 mg td)

- **Third line therapy attempt given on a case by case basis**

*Malfertheiner et al., APT 2002;16:167-180*

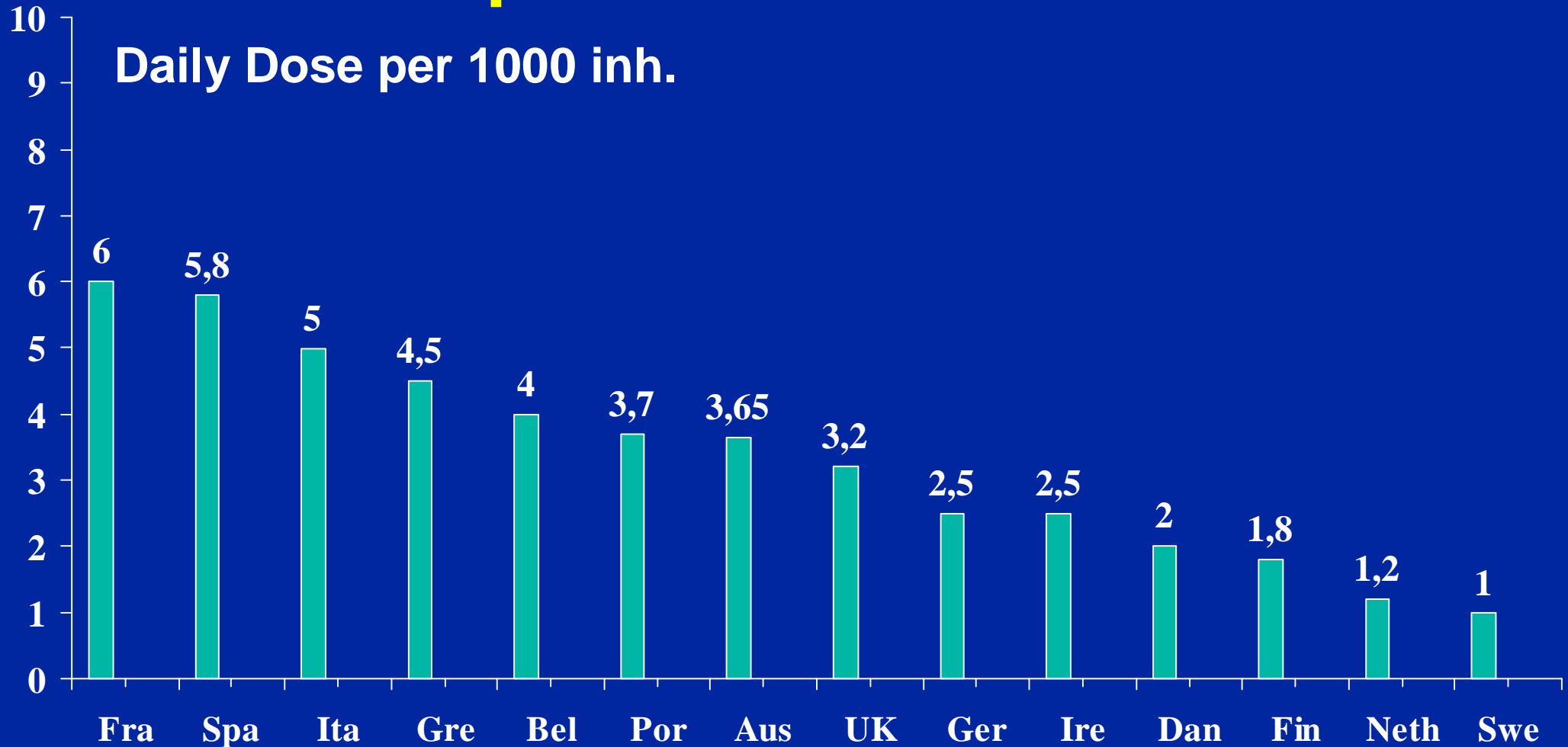
# Resistance of *H. pylori* to clarithromycin in Europe



No. of isolates tested: 1305 (mean No./Center = 59, range: 19-104)

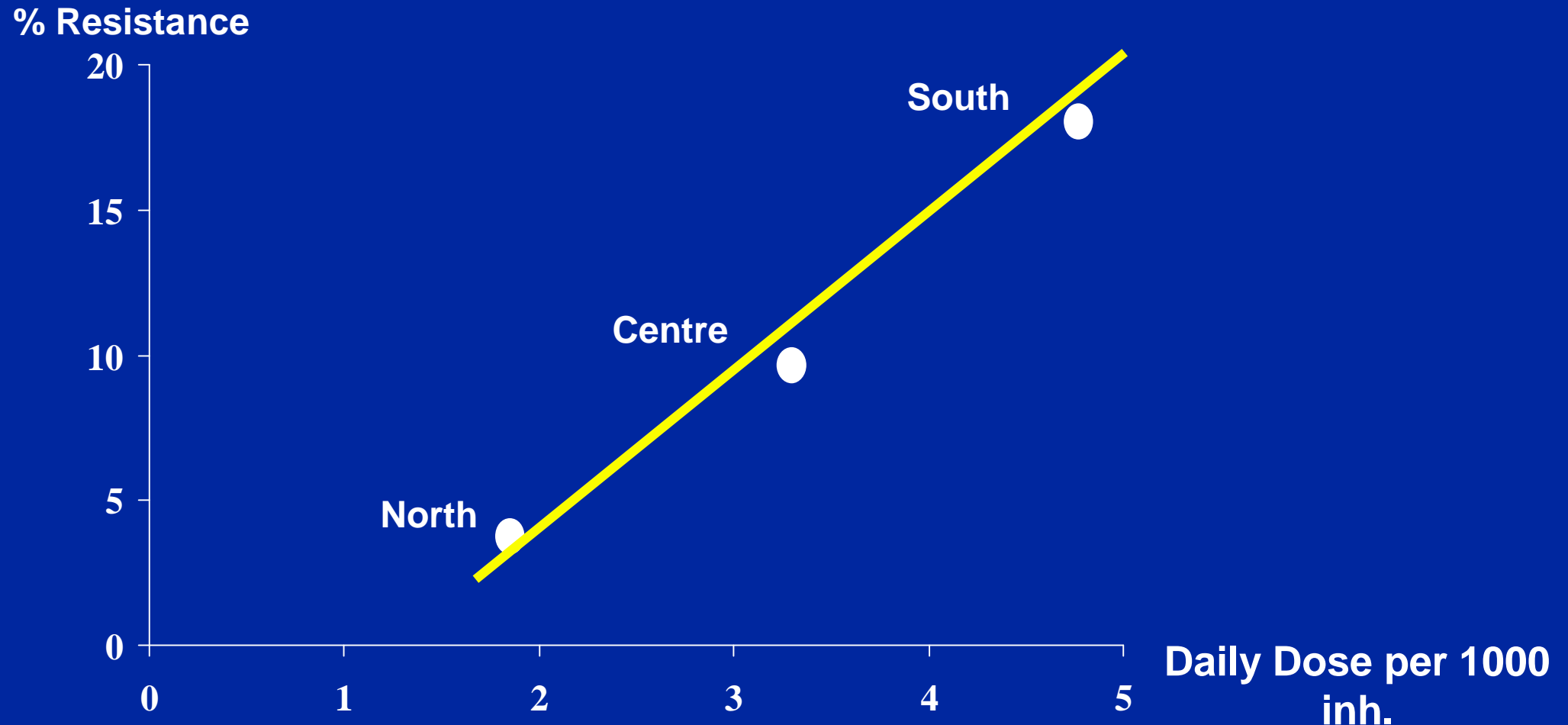
*Glupczynski et al., Eur J Clin Microbiol Inf Dis, 2002, 20: 820-3*

# Macrolide sales for outpatients in European Union - 1997



*Cars et al., Lancet 2001;357:1851-53*

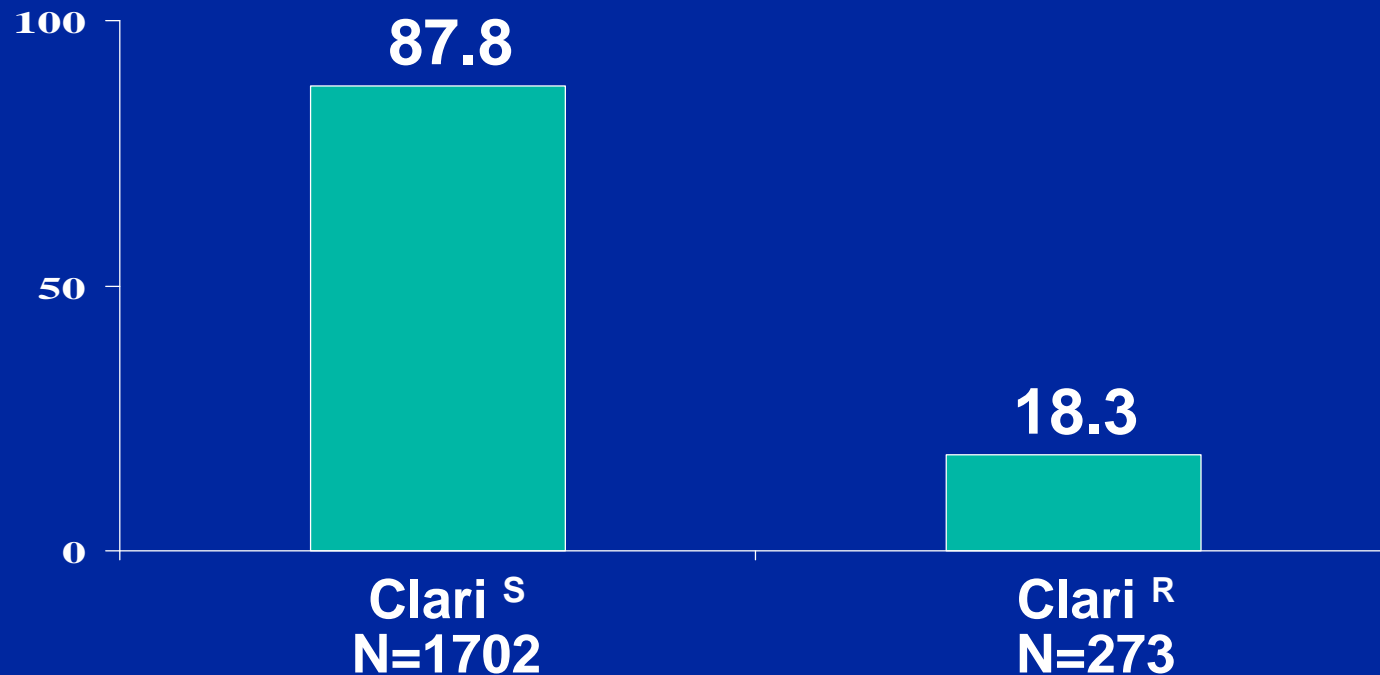
# *H. pylori* resistance to macrolides according to macrolide consumption in Europe



**Amoxicillin or metronidazole?**

# Eradication of *H. pylori* in 20 clinical trials (1999-2003) using PPI -clarithromycin-amoxicillin according to clarithromycin susceptibility

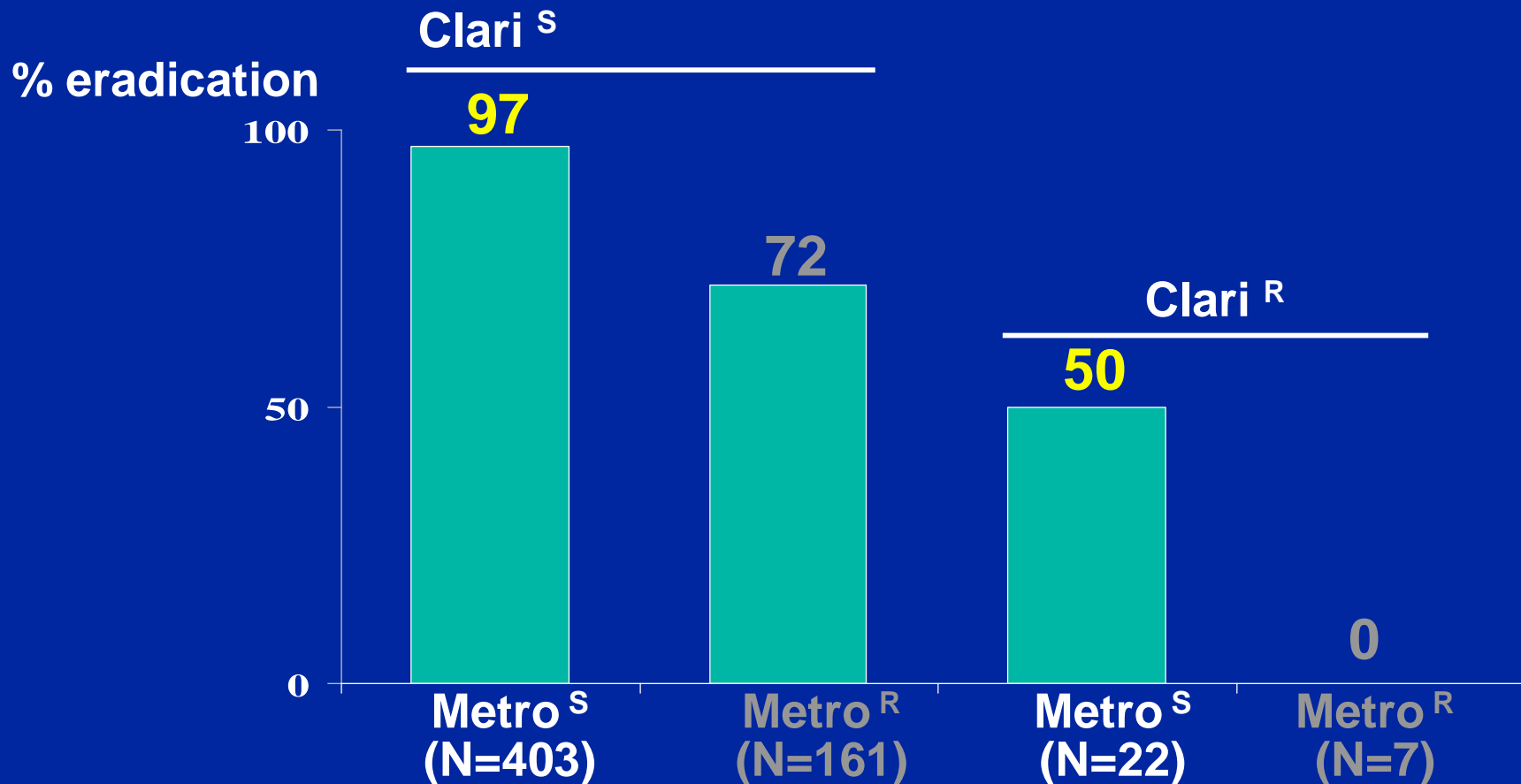
% eradication



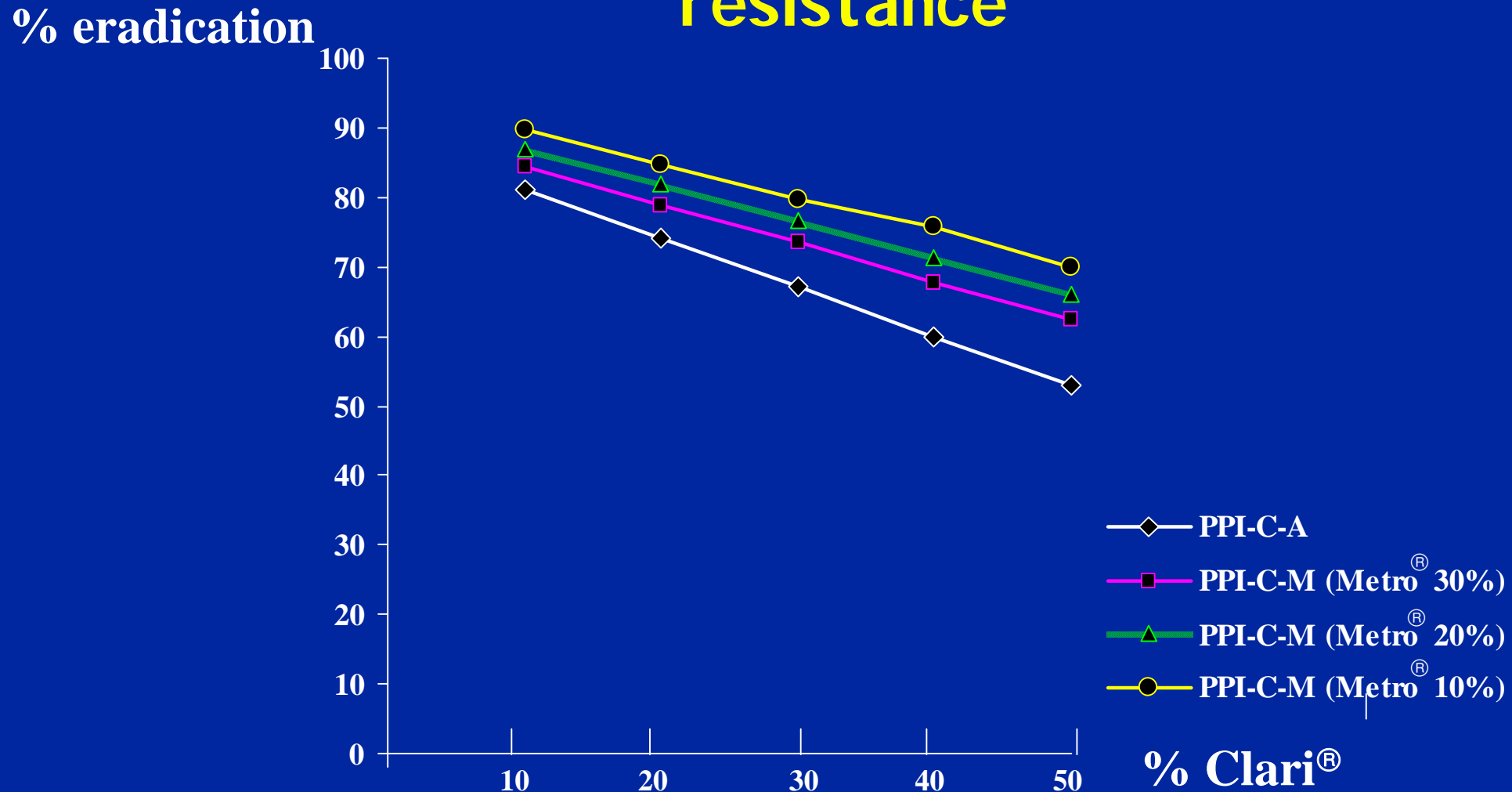
Mantel Haenszel pooled OR=24.5 [95 CI 17.2-35].  
**70% decrease** in *H. pylori* eradication if the strain is Clari<sup>R</sup>

Mégraud F., Gut 2004;53:1374-84

# Eradication of *H. pylori* in 8 clinical trials (1999-2003) using PPI -clarithromycin-metronidazole according to susceptibility to both antibiotics

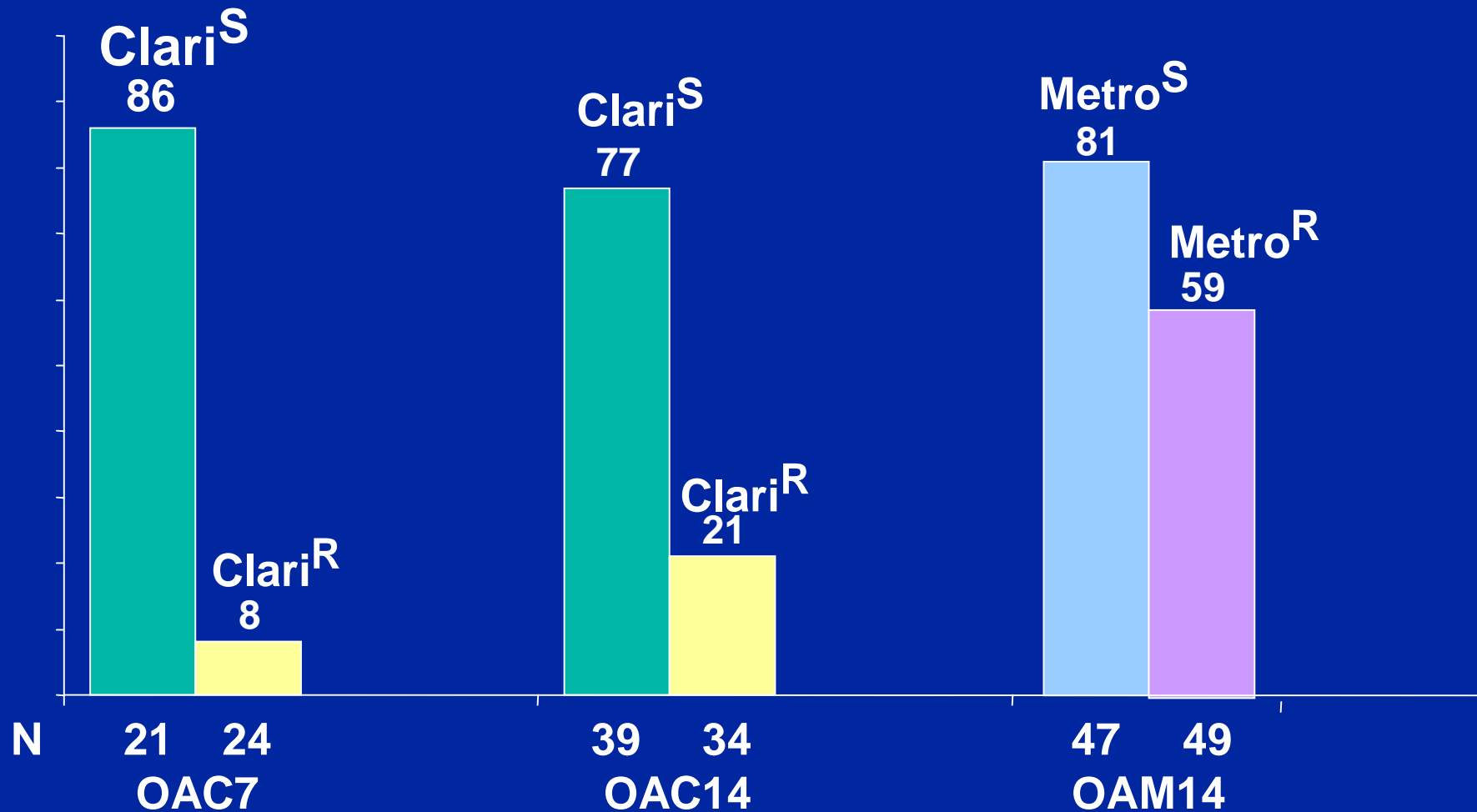


# Predicted eradication rates for 1st line triple therapies according to the level of antibiotic resistance

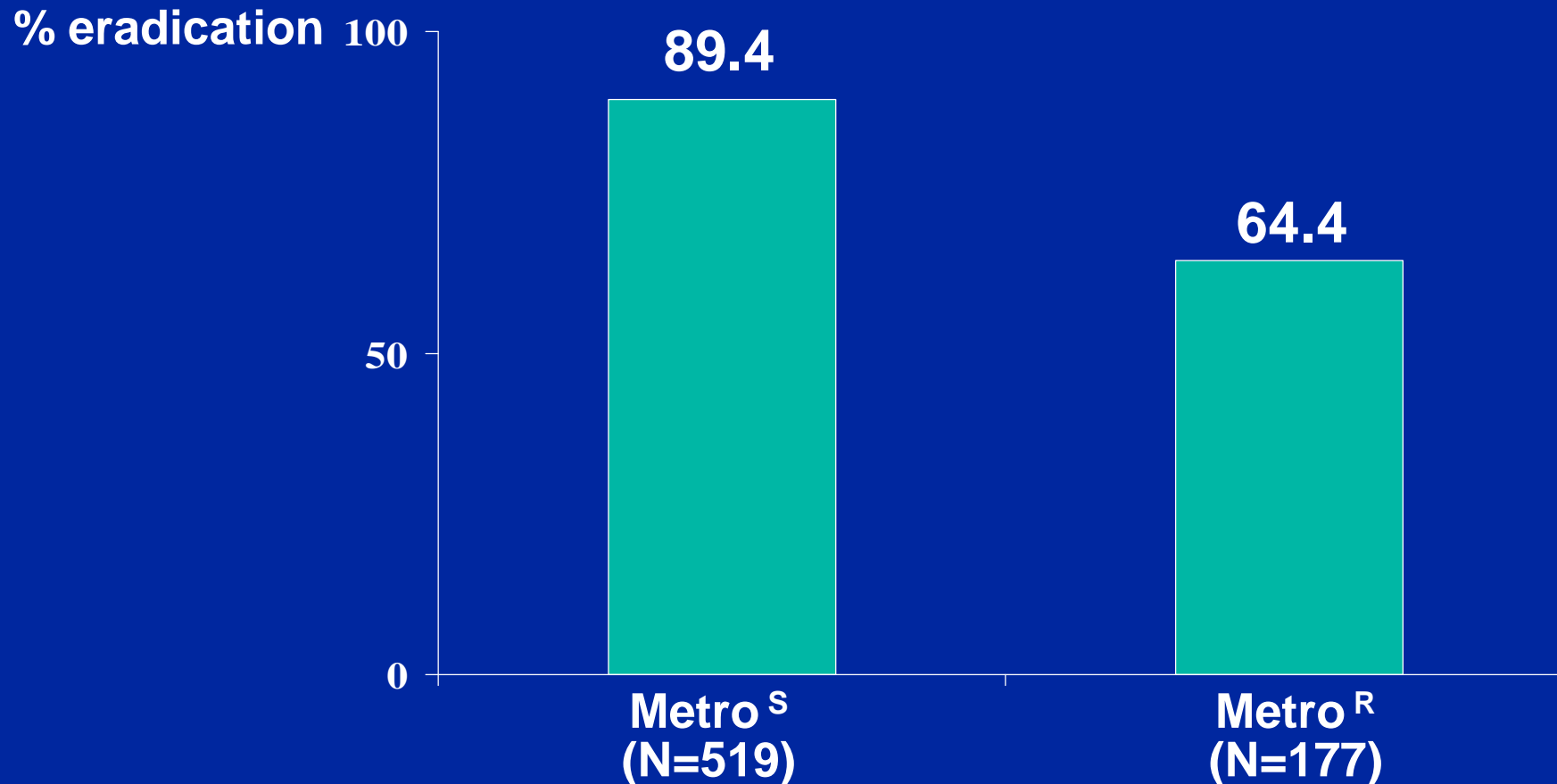


# Eradication rate according to the results of susceptibility testing in second line therapy

% eradication



# Eradication of *H. pylori* in 6 clinical trials (1999-2003) using PPI-amoxicillin-metronidazole according to metronidazole susceptibility



**25% decrease** in *H. pylori* eradication if the strain is Metro<sup>R</sup>

*Mégraud F., Gut 2004;53:1374-84*

# Conclusions

## *Limited changes since Maastricht 2-2000*

- Confirmation of the great value of the UBT and promising results of monoclonal antibody stool tests
- Role of serology under certain circumstances
- Clarithromycin based triple therapy still the leading first line treatment but in areas of high prevalence of this resistance it must be tested before use
- Metronidazole is preferred to amoxicillin except in areas of high prevalence
- Quadruple therapy can be considered as an alternative first line therapy