# *Helicobacter pylori* - associated malignancies

## Genetics, Epidemiology and Gastric Cancer Risk

Lisette G. Capelle

# *Helicobacter pylori* - associated malignancies

## Genetics, Epidemiology and Gastric Cancer Risk

Lisette G. Capelle

ISBN: 978-90-8559-074-3
Layout and print: Optima Grafische Communicatie, Rotterdam, The Netherlands
Cover: Annette Capelle

Financial support for printing this thesis was kindly given by AstraZeneca BV, Tramedico BV, Olympus Nederland B.V., Solvay Pharma BV, Roche Nederland B.V., Ferring Pharmaceuticals, and the Department of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam.

# *Helicobacter pylori*-associated malignancies:
## Genetics, Epidemiology and Gastric Cancer Risk

*Helicobacter pylori* geassocieerde maligniteiten:
Genetica, epidemiologie en maagkanker risico

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de rector magnificus

Prof. dr. H. G. Schmidt

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
vrijdag 3 september 2010 om 13:30 uur

door

**Lisette Geraldine Capelle**

geboren te Amsterdam

**PROMOTIECOMMISSIE**

| | |
|---|---|
| Promotor: | Prof. dr. E.J. Kuipers |
| Overige leden: | Prof. dr. H.W. Tilanus |
| | Prof. dr. E.W. Steyerberg |
| | Prof. dr. H. F. A. Vasen |

*Voor mijn ouders*

# Contents

## LYNCH SYNDROME



## ESOPHAGEAL SQUAMOUS CELL CARCINOMA

# Chapter 1

## General introduction and outline of the thesis

Lisette G. Capelle, Ernst J. Kuipers

Adapted from: *Peptic Ulcer Disease: Treatment and Current Clinical Practice, Hospital Health Care Europe 2008*

## INTRODUCTION

*Helicobacter pylori* is a gram-negative spiral organism that is capable of colonizing the gastric mucosa and forms the main cause of chronic active gastritis.[1] Colonization with *H. pylori* is the commonest infection worldwide, affecting at least half the world's population.[2] The prevalence of *H. pylori* varies by country, with a high prevalence in developing countries such as in Asia and Africa and a lower prevalence in Western Europe and North America.[1,3,4] This difference is already present in young children and remains throughout life. In Western countries *H. pylori* infection in children is low, whereas in various developing countries >80% of children is infected by *H. pylori* by the age of 10 years.[5,6] These variations are attributed to differences in environmental factors, such as improved hygiene and sanitation in industrialized countries.[7] However, despite the heavy colonization pressure with *H. pylori* in developing countries, some 5-10% of the population do not become infected.[8] This observation indicates that genetic factors may also play a role in *H. pylori* susceptibility, which was confirmed in a study on monozygotic and dizygotic twins that demonstrated a significantly higher concordance rate for *H. pylori* in monozygotic twins than in dizygotic twins.[9,10] However, specific host genetic factors that influence this susceptibility remain unknown. The first aim of this thesis was therefore to identify the association between specific host genetic polymorphisms and *H. pylori* infection in a genome wide association study (**Chapter 2**).

## *H. PYLORI* – ASSOCIATED MALIGNANCIES

Although the incidence of *H. pylori* infection is decreasing in Western countries, it still remains a major health problem, particularly among those above 50 years of age. *H. pylori* causes a chronic inflammation of the gastric mucosa in virtually all infected subjects.[1] This inflammation can progress to peptic ulcer disease (PUD), gastric MALT lymphoma, and pre-malignant gastric lesions. Although only a small proportion of patients with *H. pylori* will eventually develop malignant disease, the widespread high prevalence of this bacterium explains that gastric cancer remains the fourth most common cancer and second leading cause of cancer related death worldwide.[11,12] For these reasons, epidemiology and gastric cancer risk assessment in patients with progression of *H. pylori* infection to PUD, gastric MALT lymphoma, or pre-malignant gastric lesions may result in improved prognosis and a reduction in gastric cancer mortality worldwide.

## AIM

The general aim of this thesis was to evaluate epidemiological time trends and gastric adenocarcinoma risk in patients with *H. pylori*-associated malignancies such as gastric MALT lymphoma, pre-malignant gastric lesions and Lynch syndrome.

### *Peptic ulcer disease*

Although exact epidemiological data are lacking, it is estimated that at least 10% of the population suffers from PUD during lifetime. The predominant causes of PUD are *Helicobacter pylori* infection and the use of NSAIDs. Patients with a *H. pylori* infection have an estimated lifetime risk of 5-15% for PUD, for patients who use NSAIDs daily this risk is even higher. During the last decades the incidence of PUD declined due to the introduction of acid suppressive agents and the recognition of *H. pylori* as important etiologic factor for PUD. However, the admission rate for complicated ulcers, such as ulcers associated with bleeding, perforation or obstruction remained nearly constant over this period.[13] Explanations for the persistently high admission rates for complicated PUD are the increasing use of NSAIDs in particular in elderly who often also suffer from co-morbidity. This effect is enhanced by the lack of both prescription and use of proper gastroprotective treatment in many of these patients.[14,15] Since complicated ulcer disease is accompanied by high morbidity and mortality and the prevalence increases with advancing age, it is expected that this common disease will continue to have an important impact on healthcare in the coming decade.

### *Gastric MALT lymphoma*

*Helicobacter pylori* infection has increasingly been recognized in the pathogenesis of gastric Mucosa-Associated Lymphoid Tissue (MALT) lymphomas.[4,16] However, in contrast to the decreasing incidence of *H. pylori* infection and peptic ulcer disease, the incidence of gastric MALT lymphoma was reported to increase, particularly in the early nineties.[4,17-20] In these years the first link between *H. pylori* and gastric MALT lymphoma was discovered by Wotherspoon.[4] This discovery led to a major change in therapy from chemotherapy and surgery to *H. pylori* eradication. However, despite these improved treatment options, certain studies described an increased gastric adenocarcinoma risk for patients with gastric MALT lymphoma diagnosis. Moreover, an increased prevalence of pre-malignant gastric lesions and an even more rapid progression of these lesions was reported in patients with gastric MALT lymphoma.[21-24] However, most of these previous studies were small or described case series without long-term follow-up. Therefore, the prevalence of pre-malignant gastric lesions and the risk of gastric adenocarcinoma remained fairly unknown in patients with gastric MALT lymphoma.

For these reasons, the second aim of this thesis was to evaluate epidemiology and gastric cancer risk in patients with gastric MALT lymphoma in a nation-wide study with long-term of follow-up (**Chapter 3**). The third aim was to evaluate the severity of pre-malignant gastric lesions in gastric MALT lymphoma patients with a subsequent diagnosis of gastric cancer and without a subsequent diagnosis of gastric cancer to identify a subpopulation of gastric MALT lymphoma patients at high gastric cancer risk (**Chapter 4**).

### *Pre-malignant gastric lesions*

In 1992, Correa et al. demonstrated that the development of intestinal type gastric cancer occurs according to a multistep pathway.[25] In this pathway, chronic inflammation of the gastric mucosa caused by *H. pylori*, may progress through the pre-malignant stages of atrophic gastritis, intestinal metaplasia and dysplasia eventually to gastric adenocarcinoma.[25] Furthermore, various studies reported an increased gastric cancer risk in patients with pre-malignant gastric lesions.[26-29] However, best evidence for a significantly increased gastric cancer risk in patients with pre-malignant gastric lesions was provided by a previous study from de Vries et al.[30] In this nationwide cohort study in the Netherlands a gastric cancer risk of 0.8%, 1.8%, 3.9% and 32.7% for patients with atrophic gastritis, intestinal metaplasia, mild-to-moderate dysplasia and severe dysplasia respectively was demonstrated within 10 years after initial diagnosis.[30] These findings indicated that gastric cancer screening may reduce mortality and that upper gastrointestinal surveillance endoscopy needs to be considered in all patients with severe pre-malignant gastric lesions.

#### *Serological screening for pre-malignant gastric lesions*

In Japan, the implementation of a nationwide mass screening program has led to the detection of gastric cancer at early stage.[29,31-33] With regard to cost-effectiveness and burden of patients, a nationwide screening program in countries with low gastric cancer incidence seems less appropriate. In these countries a more targeted approach to detect patients at high risk of gastric cancer is required.[29] Previous studies demonstrated that such a risk profile should be based on epidemiological factors and serological screening.[29,34] Although for atrophic gastritis, serological testing for a combination of serum markers has yielded accurate results, the use of serological markers for the prediction of advanced pre-malignant gastric lesions such as intestinal metaplasia and dysplasia showed low sensitivity and specificity.[35-39] For these reasons, new markers are necessary for the prediction of patients with high gastric cancer risk.

The common denominator of these serological markers is their release by specialized cells of the stomach lining. Based on this characteristic, leptin has previously been identified as potential new serological marker for pathological conditions of the stomach.[40-42] However, whether serum leptin levels can fulfill the role of a new serological marker in gastric carcinogenesis remains unknown. Therefore, the fourth aim of this thesis was to evaluate whether

serum leptin levels can serve as a new tool to identify patients at high risk of gastric cancer (**Chapter 5**).

*Endoscopic surveillance of pre-malignant gastric lesions*

After screening, endoscopic surveillance of patients at high risk of gastric cancer could lead to early detection of patients with disease progression. However, previous studies demonstrated that current endoscopic surveillance of pre-malignant gastric lesions is discrepant with the substantial gastric cancer risk of these lesions.[30] The golden standard for diagnosing pre-malignant gastric lesions is histology of biopsy specimens. Although image quality of endoscopy has improved dramatically over the past years, endoscopic evaluation of the gastric mucosa correlates poorly with histological findings.[43-46] Therefore, a diagnosis of pre-malignant gastric lesions remains dependent on random biopsy sampling during conventional white light endoscopy. New imaging techniques are required to improve the detection of pre-malignant gastric lesions. For instance, the use of different narrow-band filters showed an improved endoscopic accuracy in detection of gastrointestinal pre-neoplastic lesions, in particular for colon and oesophagus.[47-49] Whether these endoscopic techniques also show an increased detection rate of pre-malignant gastric lesions remains unclear. The fifth aim of this thesis was therefore to compare the yield of narrow-band imaging (NBI) over conventional white light endoscopy in the surveillance of patients with advanced pre-malignant gastric lesions (**Chapter 6**).

*Histology of pre-malignant gastric lesions*

Since a previous study demonstrated that less than 2% of patients with atrophic gastritis and intestinal metaplasia will develop gastric adenocarcinoma within ten years, upper gastrointestinal surveillance endoscopy is not indicated for all patients with pre-malignant gastric lesions.[30] Preferably, patients with high gastric cancer risk should be included in a surveillance program. For that purpose, appropriate biopsy sampling at index endoscopy is essential. These biopsies can be used as most important input for risk classification, either using a broad risk classification including epidemiological, clinical and serological parameters as described previously, or using the recently proposed OLGA staging system.[34,50] This new histological staging system proposed to grade patients with gastritis into stages with corresponding gastric cancer risk.[51] Further studies showed that this new staging system provides relevant clinical information.[52,53] However, interobserver agreement for atrophic gastritis, the major parameter of the OLGA staging system is low. In contrast, previous studies have shown an excellent inter-individual agreement for the evaluation of intestinal metaplasia.[54-56] Although a histological staging system that identifies patients at high gastric cancer risk is of great clinical importance, reproducibility of such a system is necessary. A histological subclassification based on the severity and extent of intestinal metaplasia might yield more reproducible results for the identification of patients at high gastric cancer risk than a stag-

ing system based on gastritis. The sixth aim of this thesis was therefore to assess whether a staging system based on intestinal metaplasia instead of atrophic gastritis may be preferred to estimate gastric cancer risk (**Chapter 7**).

### *Gastric cancer risk in Lynch syndrome mutation carriers*

Apart from gastric MALT lymphoma and pre-malignant gastric lesions, patients diagnosed with the Lynch syndrome may also have an increased gastric cancer risk. The Lynch syndrome is caused by germline mutations in four mismatch repair genes (MLH1, MSH2, MSH6 and PMS2) and was formerly known as Hereditary Non-Polyposis Colorectal Cancer (HNPCC). The specific mutations may result in a large spectrum of different tumours that can occur during lifetime.[57-60] These in particular include colorectal cancer and endometrial cancer with a high lifetime risk, resulting in early surveillance for these types of tumours. Although gastric cancer also forms part of the Lynch syndrome tumour spectrum, clear incidence trends of gastric cancer in Lynch syndrome mutation carriers are lacking.[61-64] Moreover, the actual gastric cancer risk remains controversial in these subjects which is mainly a result of differences in study design and included populations in previous studies.[65-68] For these reasons, clear surveillance guidelines for gastric cancer are controversial or lacking in Western countries. The seventh aim of this thesis was to evaluate whether surveillance of Lynch syndrome mutation carriers is indicated in a Western population, by evaluating epidemiological time trends of gastric cancer and cumulative and relative gastric cancer risk (**Chapter 8**).

### *Esophageal squamous cell carcinoma*

Despite the role of *H. pylori* in gastric malignancies, previous studies also described a potential role of *H. pylori* in the etiology of esophageal diseases.[69] A negative association was demonstrated between *H. pylori* infection and the development of gastro-esophageal reflux diseases and the related complications such as Barrett's oesophagus or esophageal adenocarcinoma.[70-72] In line with these observations, previous studies described an increased risk of esophageal squamous cell carcinoma (ESCC) in patients with atrophic gastritis.[73-75] However, a clear explanation for this increased risk has not been provided by previous studies.

The last aim of this thesis was to examine the relation between gastric atrophy and ESCC to increase the understanding about the causality of this relationship (**Chapter 9**).

## OUTLINE OF THIS THESIS

In chapter 2 of this thesis we address the role of genetic factors in *H. pylori* susceptibility in a pilot genome-wide association study. The following chapters report about the possible long-

term consequences of *H. pylori* infection. In chapter 3 and 4 we describe the epidemiology and gastric cancer risk in patients with gastric MALT lymphoma and we further differentiate between gastric MALT lymphoma patients with and without a subsequent diagnosis of gastric cancer to evaluate the prevalence of pre-malignant gastric lesions. In chapter 5 to 7, new screening, surveillance and histological grading strategies are described for patients with pre-malignant gastric lesions. In chapter 8, the epidemiology and gastric cancer risk in Lynch syndrome mutation carriers is described and in chapter 9 the association between *H. pylori* and esophageal squamous cell carcinoma is further explored.

## REFERENCES


1 Farinha P, Gascoyne RD. Helicobacter pylori and MALT lymphoma. *Gastroenterology* 2005;128: 1579-605.

2 Parsonnet J. Helicobacter pylori: the size of the problem. *Gut* 1998;43 Suppl 1:S6-9.

3 Forman D, Graham DY. Review article: impact of Helicobacter pylori on society-role for a strategy of 'search and eradicate'. *Aliment Pharmacol Ther* 2004;19 Suppl 1:17-21.

4 Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR*, et al.* Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. *Lancet* 1991;338:1175-6.

5 Holcombe C, Omotara BA, Eldridge J*, et al.* H. pylori, the most common bacterial infection in Africa: a random serological study. *Am J Gastroenterol* 1992;87:28-30.

6 Segal I, Ally R, Mitchell H. Helicobacter pylori--an African perspective. *Qjm* 2001;94:561-5.

7 Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of Helicobacter pylori infection. *Clin Microbiol Rev* 2006;19:449-90.

8 Bardhan PK. Epidemiological features of Helicobacter pylori infection in developing countries. *Clin Infect Dis* 1997;25:973-8.

9 Malaty HM, Evans DG, Evans DJ, Jr.*, et al.* Helicobacter pylori in Hispanics: comparison with blacks and whites of similar age and socioeconomic class. *Gastroenterology* 1992;103:813-6.

10 Malaty HM, Engstrand L, Pedersen NL*, et al.* Helicobacter pylori infection: genetic and environmental influences. A study of twins. *Ann Intern Med* 1994;120:982-6.

11 Suerbaum S, Michetti P. Helicobacter pylori infection. *N Engl J Med* 2002;347:1175-86.

12 Ferlay J BF, Pisani P*, et al.* . *GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide. IARC CancerBase No 5 version 2.0*. Lyon: IARCPress. 2004.

13 Post PN, Kuipers EJ, Meijer GA. Declining incidence of peptic ulcer but not of its complications: a nation-wide study in The Netherlands. *Aliment Pharmacol Ther* 2006;23:1587-93.

14 van Leerdam ME, Vreeburg EM, Rauws EA*, et al.* Acute upper GI bleeding: did anything change? Time trend analysis of incidence and outcome of acute upper GI bleeding between 1993/1994 and 2000. *Am J Gastroenterol* 2003;98:1494-9.

15 van Soest EM, Sturkenboom MC, Dieleman JP*, et al.* Adherence to gastroprotection and the risk of NSAID-related upper gastrointestinal ulcers and haemorrhage. *Aliment Pharmacol Ther* 2007;26: 265-75.

16 Isaacson P, Wright DH. Malignant lymphoma of mucosa-associated lymphoid tissue. A distinctive type of B-cell lymphoma. *Cancer* 1983;52:1410-6.

17 Gurney KA, Cartwright RA, Gilman EA. Descriptive epidemiology of gastrointestinal non-Hodgkin's lymphoma in a population-based registry. *Br J Cancer* 1999;79:1929-34.

18 Stolte M, Bayerdorffer E, Morgner A*, et al.* Helicobacter and gastric MALT lymphoma. *Gut* 2002;50 Suppl 3:III19-24.

19 Bayerdorffer E, Miehlke S, Neubauer A*, et al.* Gastric MALT-lymphoma and Helicobacter pylori infection. *Aliment Pharmacol Ther* 1997;11 Suppl 1:89-94.

20 Severson RK, Davis S. Increasing incidence of primary gastric lymphoma. *Cancer* 1990;66:1283-7.

21 Arista-Nasr J, Jimenez-Rosas F, Uribe-Uribe N*, et al.* Pathological disorders of the gastric mucosa surrounding carcinomas and primary lymphomas. *Am J Gastroenterol* 2001;96:1746-50.

22 Driessen A, Ectors N, Creemers J*, et al.* Intestinal metaplasia in gastric malignancy: a comparison between carcinoma and lymphoma. *Eur J Gastroenterol Hepatol* 1998;10:595-600.

23 Driessen A, Ectors N, Van Cutsem E*, et al.* Different gastritis features are linked to different gastric neoplasms. *Gastroenterol Clin Biol* 1999;23:747-53.

24 Lamarque D, Levy M, Chaumette MT, *et al.* Frequent and rapid progression of atrophy and intestinal metaplasia in gastric mucosa of patients with MALT lymphoma. *Am J Gastroenterol* 2006;101: 1886-93.

25 Correa P. Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 1992;52:6735-40.

26 Filipe MI, Munoz N, Matko I, *et al.* Intestinal metaplasia types and the risk of gastric cancer: a cohort study in Slovenia. *Int J Cancer* 1994;57:324-9.

27 Whiting JL, Sigurdsson A, Rowlands DC, *et al.* The long term results of endoscopic surveillance of premalignant gastric lesions. *Gut* 2002;50:378-81.

28 El-Zimaity HM, Ramchatesingh J, Saeed MA, *et al.* Gastric intestinal metaplasia: subtypes and natural history. *J Clin Pathol* 2001;54:679-83.

29 de Vries AC, Haringsma J, Kuipers EJ. The detection, surveillance and treatment of premalignant gastric lesions related to Helicobacter pylori infection. *Helicobacter* 2007;12:1-15.

30 de Vries AC, van Grieken NC, Looman CW, *et al.* Gastric cancer risk in patients with premalignant gastric lesions: a nationwide cohort study in the Netherlands. *Gastroenterology* 2008;134:945-52.

31 Hosokawa O, Miyanaga T, Kaizaki Y, *et al.* Decreased death from gastric cancer by endoscopic screening: association with a population-based cancer registry. *Scand J Gastroenterol* 2008;43: 1112-5.

32 Lee KJ, Inoue M, Otani T, *et al.* Gastric cancer screening and subsequent risk of gastric cancer: a large-scale population-based cohort study, with a 13-year follow-up in Japan. *Int J Cancer* 2006; 118:2315-21.

33 Oshima A, Hirata N, Ubukata T, *et al.* Evaluation of a mass screening program for stomach cancer with a case-control study design. *Int J Cancer* 1986;38:829-33.

34 de Vries AC, Haringsma J, de Vries RA, *et al.* The use of clinical, histologic, and serologic parameters to predict the intragastric extent of intestinal metaplasia: a recommendation for routine practice. *Gastrointest Endosc* 2009;70:18-25.

35 Vaananen H, Vauhkonen M, Helske T, *et al.* Non-endoscopic diagnosis of atrophic gastritis with a blood test. Correlation between gastric histology and serum levels of gastrin-17 and pepsinogen I: a multicentre study. *Eur J Gastroenterol Hepatol* 2003;15:885-91.

36 Storskrubb T, Aro P, Ronkainen J, *et al.* Serum biomarkers provide an accurate method for diagnosis of atrophic gastritis in a general population: The Kalixanda study. *Scand J Gastroenterol* 2008:1-8.

37 Watabe H, Mitsushima T, Yamaji Y, *et al.* Predicting the development of gastric cancer from combining Helicobacter pylori antibodies and serum pepsinogen status: a prospective endoscopic cohort study. *Gut* 2005;54:764-8.

38 Sipponen P, Ranta P, Helske T, *et al.* Serum levels of amidated gastrin-17 and pepsinogen I in atrophic gastritis: an observational case-control study. *Scand J Gastroenterol* 2002;37:785-91.

39 Dinis-Ribeiro M, da Costa-Pereira A, Lopes C, *et al.* Validity of serum pepsinogen I/II ratio for the diagnosis of gastric epithelial dysplasia and intestinal metaplasia during the follow-up of patients at risk for intestinal-type gastric adenocarcinoma. *Neoplasia* 2004;6:449-56.

40 Bado A, Levasseur S, Attoub S, *et al.* The stomach is a source of leptin. *Nature* 1998;394:790-3.

41 Francois F, Roper J, Goodman AJ, *et al.* The association of gastric leptin with oesophageal inflammation and metaplasia. *Gut* 2008;57:16-24.

42 Zhang Y, Proenca R, Maffei M, *et al.* Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994;372:425-32.

43 Redeen S, Petersson F, Jonsson KA, *et al.* Relationship of gastroscopic features to histological findings in gastritis and Helicobacter pylori infection in a general population sample. *Endoscopy* 2003;35:946-50.

44 Lin BR, Shun CT, Wang TH, *et al.* Endoscopic diagnosis of intestinal metaplasia of stomach--accuracy judged by histology. *Hepatogastroenterology* 1999;46:162-6.

45 Sauerbruch T, Schreiber MA, Schussler P, *et al.* Endoscopy in the diagnosis of gastritis. Diagnostic value of endoscopic criteria in relation to histological diagnosis. *Endoscopy* 1984;16:101-4.

46 Meshkinpour H, Orlando RA, Arguello JF, *et al.* Significance of endoscopically visible blood vessels as an index of atrophic gastritis. *Am J Gastroenterol* 1979;71:376-9.

47 Uedo N, Ishihara R, Iishi H, *et al.* A new method of diagnosing gastric intestinal metaplasia: narrow-band imaging with magnifying endoscopy. *Endoscopy* 2006;38:819-24.

48 Nakayoshi T, Tajiri H, Matsuda K, *et al.* Magnifying endoscopy combined with narrow band imaging system for early gastric cancer: correlation of vascular pattern with histopathology (including video). *Endoscopy* 2004;36:1080-4.

49 East JE, Tan EK, Bergman JJ, *et al.* Meta-analysis: narrow band imaging for lesion characterization in the colon, oesophagus, duodenal ampulla and lung. *Aliment Pharmacol Ther* 2008;28:854-67.

50 Rugge M, Genta RM. Staging gastritis: an international proposal. *Gastroenterology* 2005;129:1807-8.

51 Rugge M, Correa P, Di Mario F, *et al.* OLGA staging for gastritis: a tutorial. *Dig Liver Dis* 2008;40: 650-8.

52 Rugge M, Meggio A, Pennelli G, *et al.* Gastritis staging in clinical practice: the OLGA staging system. *Gut* 2007;56:631-6.

53 Satoh K, Osawa H, Yoshizawa M, *et al.* Assessment of atrophic gastritis using the OLGA system. *Helicobacter* 2008;13:225-9.

54 Chen XY, van der Hulst RW, Bruno MJ, *et al.* Interobserver variation in the histopathological scoring of Helicobacter pylori related gastritis. *J Clin Pathol* 1999;52:612-5.

55 el-Zimaity HM, Graham DY, al-Assi MT, *et al.* Interobserver variation in the histopathological assessment of Helicobacter pylori gastritis. *Hum Pathol* 1996;27:35-41.

56 Guarner J, Herrera-Goepfert R, Mohar A, *et al.* Interobserver variability in application of the revised Sydney classification for gastritis. *Hum Pathol* 1999;30:1431-4.

57 Vasen HF. Review article: The Lynch syndrome (hereditary nonpolyposis colorectal cancer). *Aliment Pharmacol Ther* 2007;26 Suppl 2:113-26.

58 Peltomaki P, Vasen H. Mutations associated with HNPCC predisposition -- Update of ICG-HNPCC/INSiGHT mutation database. *Dis Markers* 2004;20:269-76.

59 Lynch HT, Lynch JF. Lynch syndrome: history and current status. *Dis Markers* 2004;20:181-98.

60 Lynch HT, Smyrk TC, Watson P, *et al.* Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: an updated review. *Gastroenterology* 1993;104:1535-49.

61 Watson P, Lynch HT. Extracolonic cancer in hereditary nonpolyposis colorectal cancer. *Cancer* 1993;71:677-85.

62 Watson P, Lynch HT. The tumor spectrum in HNPCC. *Anticancer Res* 1994;14:1635-9.

63 Watson P, Vasen HF, Mecklin JP, *et al.* The risk of extra-colonic, extra-endometrial cancer in the Lynch syndrome. *Int J Cancer* 2008;123:444-9.

64 Maul JS, Warner NR, Kuwada SK, *et al.* Extracolonic cancers associated with hereditary nonpolyposis colorectal cancer in the Utah Population Database. *Am J Gastroenterol* 2006;101:1591-6.

65 Vasen HF, Moslein G, Alonso A, *et al.* Guidelines for the clinical management of Lynch syndrome (hereditary non-polyposis cancer). *J Med Genet* 2007;44:353-62.

66 Park YJ, Shin KH, Park JG. Risk of gastric cancer in hereditary nonpolyposis colorectal cancer in Korea. *Clin Cancer Res* 2000;6:2994-8.
67 Goecke T, Schulmann K, Engel C, *et al.* Genotype-phenotype comparison of German MLH1 and MSH2 mutation carriers clinically affected with Lynch syndrome: a report by the German HNPCC Consortium. *J Clin Oncol* 2006;24:4285-92.
68 Aarnio M, Sankila R, Pukkala E, *et al.* Cancer risk in mutation carriers of DNA-mismatch-repair genes. *Int J Cancer* 1999;81:214-8.
69 Malfertheiner P, Megraud F, O'Morain C, *et al.* Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut* 2007;56:772-81.
70 Wu AH, Crabtree JE, Bernstein L, *et al.* Role of Helicobacter pylori CagA+ strains and risk of adenocarcinoma of the stomach and esophagus. *Int J Cancer* 2003;103:815-21.
71 Vicari JJ, Peek RM, Falk GW, *et al.* The seroprevalence of cagA-positive Helicobacter pylori strains in the spectrum of gastroesophageal reflux disease. *Gastroenterology* 1998;115:50-7.
72 Chow WH, Blaser MJ, Blot WJ, *et al.* An inverse relation between cagA+ strains of Helicobacter pylori infection and risk of esophageal and gastric cardia adenocarcinoma. *Cancer Res* 1998;58: 588-90.
73 Iijima K, Koike T, Abe Y, *et al.* Extensive gastric atrophy: an increased risk factor for superficial esophageal squamous cell carcinoma in Japan. *Am J Gastroenterol* 2007;102:1603-9.
74 Ye W, Held M, Lagergren J, *et al.* Helicobacter pylori infection and gastric atrophy: risk of adenocarcinoma and squamous-cell carcinoma of the esophagus and adenocarcinoma of the gastric cardia. *J Natl Cancer Inst* 2004;96:388-96.
75 McColl KE. Helicobacter pylori and oesophageal cancer--not always protective. *Gut* 2007;56:457-9.

# Chapter 2

## The identification of host genetic polymorphisms for *H. pylori* infection: a genome wide association study

Lisette G. Capelle[1], Annemarie C. de Vries[1], Lisette Stolk[2,3], Michael M. P. J. Verbiest[2,3], Leon M. G. Moons[1], Joyce. B. van Meurs[2,3], André G. Uitterlinden[2,3], Ernst J. Kuipers[1,2]

*[1]Departments of Gastroenterology and Hepatology, [2]Internal Medicine, and [3]Epidemiology, Erasmus MC University Medical Center, Rotterdam*

## ABSTRACT

*Background: Helicobacter pylori* infection affects at least half the world's population. Important risk factors for acquiring *H. pylori* are associated with low socioeconomic status. Genetic factors also seem to play a role in *H. pylori* susceptibility, as illustrated by a previous observation that dizygotic twins had a lower concordance for *H. pylori* status than monozygotic twins. However, specific associations between genetic polymorphisms and the host that influence the acquisition of *H. pylori* are unknown. We therefore performed a genome wide association study (GWAS) to identify hypothetical associations between host genetic polymorphisms and *H. pylori* infection.

*Methods:* We performed a Genome Wide Association analysis using 900,000 single nucleotide polymorphisms (SNPs) in a discovery cohort of 509 Caucasian women in the Rotterdam Study. We then evaluated the 40 most promising SNPs (association with *H. pylori*-status $p<10^{-4}$) in a replication cohort of 496 subjects from the Netherlands. Serum *H. pylori* antibodies were measured using commercial enzyme immunoassays.

*Results:* In total, 169 *H. pylori*-positive women and 340 *H. pylori*-negative women (median age 62 years, SD) were included in the discovery cohort. The replication cohort (M/F 250/246, 96% Caucasians, age 55 years (SD 15.1)) consisted of 108 *H. pylori*-positive cases and 388 *H. pylori*-negative controls. We identified compelling genome-wide evidence for an association between *H. pylori* infection and one SNP (rs17015126 on chromosome 2; minor alle frequency (MAF) 0.07; combined p-value=$2.8 \times 10^{-7}$). In addition to this SNP, there was a suggestive genome-wide association for two other SNPs (rs1816653 on chromosome 2 and rs1939842 on chromosome 11; MAF 0.08 and MAF 0.44 and combined p-values $p=2.6 \times 10^{-5}$ and $p=2.9 \times 10^{-5}$, respectively). All three SNPs reside in unannotated regions of the chromosome and no genes were identified in the LD blocks of the SNPs. The nearest gene for both rs17015126 and rs1816653 on chromosome 2 is the leucine-rich repeat transmembrane neuronal 4 (LRRTM4) gene at a distance of >700kb. For rs1939842 the nearest gene is the loss of heterozygosity LOH11CR2A gene at a distance of 19 kb.

*Conclusions:* Although the precise identity of the underlying loci of the genome-wide associated SNPs remains elusive and the function of the nearest genes is uncertain, our study provides compelling evidence for the existence of at least one genetic region (rs17015126 on chromosome 2) that may play a role in *H. pylori* susceptibility.

## INTRODUCTION

*Helicobacter pylori* infection is the commonest chronic bacterial infection worldwide and affects at least half the world's population.[1] Although in industrialised countries the prevalence is decreasing, *H. pylori* colonization still remains common in these countries, particularly among those above 50 years of age.[1-4]

*H. pylori* causes in virtually all infected subjects a chronic inflammation of the gastric mucosa, which can progress to peptic ulcer disease, and in some 3% to gastric adenocarcinoma or mucosa-associated lymphoid tissue (MALT) lymphoma.[3, 5] Why only this small proportion of patients develops malignant disease and others do not, has been widely investigated. The majority of these previous studies have focused on the correlation between *H. pylori* and its related diseases. It is now clear that among other factors, host and bacterial genetic polymorphisms play an important role in *H. pylori* infection and gastric cancer risk.[2] However, relatively few studies have focused on the potential role of genetic factors in the acquisition and persistence of *H. pylori*.

The rate of acquisition varies between developing and industrialised countries. The prevalence of *H. pylori* infection in industrialised countries is low, in particular in children. In contrast, more than 90% of children in various developing countries are infected by *H. pylori* by the age of 10 years, and colonization with multiple strains is common.[6-9] Nevertheless, some 5-10% of the population does not become colonized with *H. pylori* infection despite apparent heavy colonization pressure.[10] This observation indicates that genetic factors may play a role in *H. pylori* susceptibility. The best evidence that genetic factors influence the susceptibility to *H. pylori* was reported by Malaty et al in a seminal paper demonstrating that the proband-wise concordance rate for *H. pylori* infection was significantly higher in monozygotic than in dizygotic twin pairs, with a heritability estimate of 57%.[11]

Although these data demonstrate that host genetic factors influence the acquisition of *H. pylori*, the underlying specific associations remain unclear. As genetic polymorphisms play an important role in *H. pylori* and its related clinical diseases, we assumed that specific host genetic polymorphism also influence the susceptibility to *H. pylori*. Therefore, we undertook a pilot Genome Wide Association Study (GWAS) to identify the association between host genetic polymorphisms and *H. pylori* infection and we replicated the associated genetic polymorphisms to confirm the association.

## METHODS

### *Patient selection*

This study was based on two cohorts. In the discovery cohort, a Genome Wide Association study was performed in 509 participants, all of whom were selected from the Rotterdam Study Population. A detailed description of the design and objective of the Rotterdam Study has been described elsewhere.[12] Briefly, the Rotterdam Study is a population based-prospective study of 7983 subjects aged 55 years and older residing in Ommoord, a suburb of Rotterdam, that aims to assess the occurrence and determinants of chronic diseases. The 509 selected participants were unrelated women aged between 60 and 75 years of Caucasian European ancestry.

In the replication cohort, which consisted of 496 participants, subjects > 18 years were included, who were identified through general practitioner centers in Rotterdam. Subjects with a history of a gastrointestinal malignancy were excluded. All participants provided written informed consent.

### *Serologic markers*

Serum was collected from all subjects. The samples were collected in serum tubes and stored in aliquots at -80°C until analysis. *H. pylori* antibodies were measured using commercial enzyme immunoassays (Orion Diagnostica). The test was performed according to the instructions of the manufacturers.

### *Sequenom iPLEX and Taqman Allelic Discrimination genotyping*

Genomic DNA was extracted from serum samples to standard procedures. 1-2 ng genomic DNA was dispensed into 384-wells plates using a Caliper Sciclone ALH3000 pipetting robot (Caliper LS, Mountain View, CA, USA). Genotyping was done using Sequenom iPLEX genotyping and Taqman Allelic Discrimination.

Multiplex PCR assays were designed for the Sequenom iPLEX genotying using Assay Designer on the website (https://mysequenom.com/tools/genotyping/default.aspx). For this, sequences containing the SNP site and at least 100 bp of flanking sequence on either side of the SNP were used. Briefly, 2 ng genomic DNA was amplified in a 5 ul reaction containing 1 × Taq PCR buffer (Sequenom), 2 mM $MgCl_2$, 500 uM each dNTP, 100 nM each PCR primer, 0.5 U Taq (Sequenom). The reaction was incubated at 94°C for 4 minutes followed by 45 cycles of 94°C for 20 seconds, 56°C for 30 seconds, 72°C for 1 minute, followed by 3 minutes at 72°C. Excess dNTPs were then removed from the reaction by incubation with 0.3 U shrimp alkaline phosphatase (Sequenom) at 37°C for 40 minutes followed by 5 minutes at 85°C to deactivate

the enzyme. Single primer extension over the SNP was carried out in a final concentration of between 0.731 uM and 1.462 uM for each extension primer (depending on the mass of the probe), iPLEX termination mix (Sequenom), 10x iPLEX Buffer Plus and iPLEX enzyme (Sequenom) and cycled using the following program; 94°C for 30 seconds followed by 94°C for 5 seconds, 5 cycles of 52°C for 5 seconds, and 80°C for 5 seconds, the last three steps were repeated 40 times, then 72°C for 3 minutes. The reaction was then desalted by addition of 6 mg clear resin (Sequenom) followed by mixing (15 minutes) and centrifugation (5 min, 3,000rpm) to settle the contents of the tube. The extension product was then spotted onto a 384 well spectroCHIP using the SEQUENOM MassARRAY Nanodispenser RS1000 before analysis on the MassARRAY Compact System (Sequenom). Data collection was performed using SpectroACQUIRE 3.3.1.13 and clustering was called using TYPER Analyzer 4.0.3.18 (Sequenom). Additionally, to ensure data quality, genotypes for each subject were also checked manually.

Genotypes for rs6861203, rs942871, and rs1816653 were generated using Taqman Allelic Discrimination (Applied Biosystems Inc., Foster City, CA, USA). All assays were available at www.appliedbiosystems.com as pre-designed assays. The PCR reaction mixture included 1-2 ng of genomic DNA in a 2 µl volume and the following reagents: FAM and VIC probes (200 nM), primers (0.9 uM), 2x Taqman PCR master mix (Applied Biosystems Inc., Foster City, CA, USA). Reagents were dispensed in a 384-well plate using the Deerac Equator NS808 (Deerac Fluidics, Dublin, Ireland). PCR cycling reaction were performed in 384 wells PCR plates in an ABI 9700 PCR system (Applied Biosystems Inc., Foster City, CA, USA) and consisted of initial denaturation for 15 minutes at 95° C, and 40 cycles with denaturation of 15 seconds at 95° C and annealing and extension for 60 seconds at 60° C. Results were analysed by the ABI Taqman 7900HT using the sequence detection system 2.22 software (Applied Biosystems Inc., Foster City, CA, USA).

### *Statistical analysis*

For quality control all SNPs with a call rate <97.5%, and SNPs deviating from Hardy-Weinberg equilibrium with a p<0.00001, were excluded from the analysis. Odds Ratios (ORs) and p-values were calculated using PLINK.[13] Inclusion criteria for replication were: 1. an association with *H. pylori* infection with a p value of $< 1x10^{-4}$; 2. an $r^2 < 0.9$; and 3. a minor allele frequency (MAF) of ≥ 0.05. Odds ratios for each replicated SNP were estimated using logistic regression.

SNPs that showed an association with *H. pylori* with a p-value ≤ 0.05 in the replication cohort, were defined as 'top associated SNPs'. For these SNPs, Odds ratios and p-values were combined in a fixed effects meta-analysis and the association to *H. pylori* for these particular SNPs was evaluated for the total cohort (n=1005) using Comprehensive meta-analysis 2.0 (Biostat, Englewood, NJ, USA).

## RESULTS

In total, 509 subjects were included in the discovery cohort and 496 subjects were included in the replication cohort. Subject characteristics of the discovery and replication cohort are shown in Table 1. Significantly more females were included in the discovery cohort in comparison to the replication cohort ($p<0.001$). Overall, the discovery cohort consisted of 169 *H. pylori*-positive women and 340 *H. pylori*-negative women whereas the replication cohort consisted of 108 *H. pylori*-positive subjects and 388 *H. pylori*-negative subjects.

**Table 1.** Baseline characteristics of the discovery and replication cohort

| | Discovery cohort | | Replication cohort | |
|---|---|---|---|---|
| | n | % | n | % |
| Total | 509 | | 496 | |
| Gender | | | | |
| - Male | - | - | 250 | 50 |
| - Female | 509 | 100 | 246 | 50 |
| Median age (SD) | 62 | | 55 (15.1) | |
| Ethnicity | | | | |
| - Caucasians | 509 | 100 | 478 | 96 |
| - Non-Caucasians | - | - | 18 | 4 |
| *H. pylori* | | | | |
| - Positive | 169 | 33 | 108 | 22 |
| - Negative | 340 | 67 | 288 | 88 |

### *SNP detection*

After quality control, 425,174 SNPs were available from the Affymetrix SNP data and 535,456 SNPs from the Illumina beadarray. Figure 1 shows the Manhattan plots for the Affymetrix data (Figure 1a) and Illumina data (Figure 1b). In total, 30 SNPs on the Illumina array and 45 SNPs in the Affymetrix dataset showed a p-value of $< 1x10^{-4}$ corresponding to 38 different loci with 12 loci having multiple significant SNPs. From these 75 SNPs, 24 SNPs were excluded since the $r^2$ was $\geq 0.9$, of the remaining 51 SNPs, 9 SNPs had a MAF of $\leq 0.05$.

In total, 42 SNPs were included for replication. Thirty-nine SNPs were genotyped using the Sequenom Assay and three SNPs using the Taqman Assay in the 496 subjects included in the replication cohort. Two SNPs failed genotyping, for the remaining 40 SNPs odds ratios and p-values were evaluated.

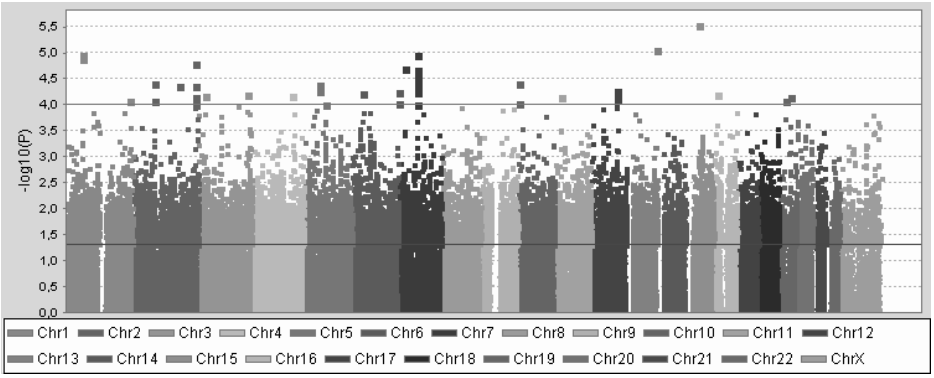


**Figure 1a.** Manhattan plot of Affymetrix data: Association to *H. pylori* of 425,174 SNPs
Legend: Red line: p value = 1 x 10-4; Blue line: p value = 0.05.

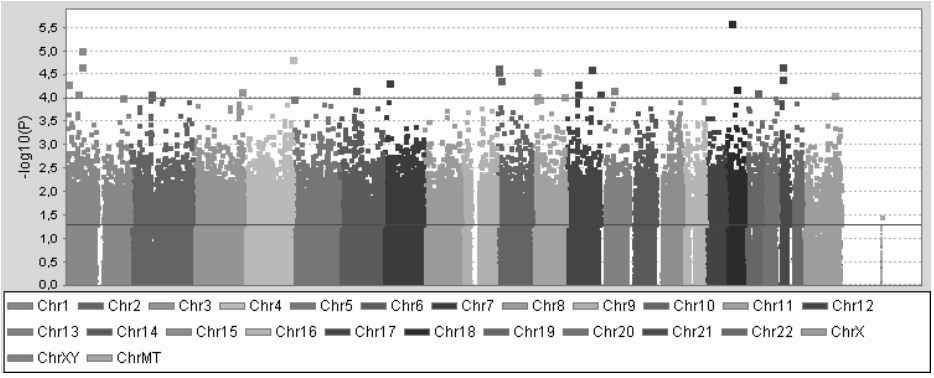


**Figure 1b:** Manhattan plot of Illumina data: Association to *H. pylori* of 535,456 SNPs
Legend: Red line: p value = 1 x 10-4; Blue line: p value = 0.05.

### *Top-associated SNPs*

Three SNPs in two loci demonstrated a significant association with *H. pylori* infection in the replication cohort ($p \leq 0.05$) (Table 2). All resided in unannotated regions, two on chromosome 2 (rs17015126 and rs1816653) and one on chromosome 11 (rs1939842). For these SNPs combined p-values and odds ratios were calculated for the total cohort (n=1005). All three SNPs showed suggestive genome-wide associations with *H. pylori* with an OR of 2.5 (95% CI 1.7-3.5; p = 2.8 x 10-7) for rs17015126, an OR of 2.7 (95% CI 1.7-4.4; p = 2.6 x 10-5) for rs1816653, respectively 0.6 (95% CI 0.5-0.8; p = 2.9 x 10-5) for rs1939842 (Table 2).

The nearest genes and genes within the LD-blocks for the two different loci were evaluated. SNPs rs17015126 and rs1816653 on chromosome 2 are located >700kb from the leucine rich repeat transmembrane neuronal 4 (LRRTM4) gene. For rs1939842, the nearest genes were the loss of heterozygosity (LOH) LOH11CR2A gene at a distance of 19 kb and the olfactory receptor (OR) genes; OR10G4, OR10G7, OR10G8 and OR10G9 at a distance of 100 to 200kb.

**Table 2.** Top associated SNPs

| | | | | Discovery cohort | | | Replication cohort | | | Overall* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SNP | Chromo-some | Position | Minor allele | MAF | OR (95% CI) | p-value | MAF | OR (95% CI) | p-value | OR (95% CI) | p-value |
| rs17015126 | 2 | 78432575 | A | 0.07 | 2.6 (1.7-4.2) | 3.8 x 10-5 | 0.05 | 2.2 (1.3-3.8) | 2.3x10-3 | 2.5 (1.7-3.5) | 2.8 x 10-7 |
| rs1816653 | 2 | 78330723 | C | 0.08 | 3.6 (1.8-7.0) | 8.7 x 10-5 | 0.03 | 2.1 (1.1-4.1) | 2.3 x 10-2 | 2.7 (1.7-4.4) | 2.6 x 10-5 |
| rs1939842 | 11 | 123471842 | C | 0.44 | 0.6 (0.4-0.7) | 8.7 x 10-5 | 0.38 | 0.7 (0.5-1.0) | 5.4 x 10-2 | 0.6 (0.5-0.8) | 2.9 x 10-5 |

Legend: MAF: Minor Allele Frequency, OR: Odds Ratio, *Overall: OR and p-value for an association with *H. pylori* in the total cohort (n=1005).

## DISCUSSION

Previous studies have investigated environmental and virulence factors for *H. pylori* susceptibility and its related diseases for years. However, for genetic factors that influence *H. pylori* susceptibility evidence is lacking. Moreover, it seemed impossible to separate genetic factors from environmental factors. Therefore specific associations between polymorphisms of the host and susceptibility to *H. pylori* are unknown. Our study shows for the first time considerable genome wide evidence for an association between host genetic factors and *H. pylori* susceptibility.

The two associated loci have not been described in previous studies concerning H. pylori and host genetics. These studies rarely focused on infection susceptibility, but mostly on disease outcome in the presence of infection. The majority of these studies investigated the link between SNPs in genes that regulate the inflammatory responses against bacteria, in particular genes that may have an effect on the unique features of *H. pylori*, i.e. flagella to swim in mucus, tight adhesions that bind to epithelial cells and neutralizing of gastric acid by urease.[2, 14] For instance, previous studies described that *H. pylori*-positive individuals with SNPs in genes encoding for IL1 have been at increased risk of developing hypochlorhydria, atrophic gastritis and eventually gastric cancer.[2, 15, 16] Besides this increased risk, IL1 polymorphisms were also reported to contribute to increased host susceptibility to *H. pylori* in a large cohort of Chinese adults.[14] The investigators hypothesized that this increased susceptibility was due to the reduced gastric acid secretion and probably improved spread and distribution of *H. pylori* throughout the stomach.[14] In contrast, a prospective study on *H. pylori* susceptibility in Jamaican mothers and children demonstrated that of 17 loci identified in 11 genes only the polymorphism at IL1a correlated with a lower risk of *H. pylori* in Jamaican children.[17] Since we investigated the role of genetic factors in *H. pylori* susceptibility in a genome wide manner without a focus limited to specific SNPs, our findings may result in new insights in basic research for susceptibility to *H. pylori* infection.

The function of the two newly discovered loci remains less clear. The loss of heterozygosity (LOH) gene near rs1939842 is located on chromosome 11 in the 11q23-q24 region. This

region has been identified as a region frequently deleted in a variety of tumours, including breast, lung and ovarian tumours, which suggests the existence of a tumour suppressor gene at this locus.[18] None of the previous studies described an association between this region and *H. pylori* infection or gastric cancer.

Other genes near rs1939842 were genes encoding for olfactory receptors (OR). At present, 390 putative, functional, protein-encoding olfactory receptor (OR) genes have been described. In addition, some 465 OR pseudogenes have been identified, which are located on multiple chromosomes, but so far mainly without identified function.[19] In addition, little is known about the function of olfactory receptors in the gut and to our knowledge, none of the studies on olfactory receptor genes described an association between these genes and *H. pylori* susceptibility. Both rs17015126 as well as rs1816653 were located >700kb from the leucine rich repeat transmembrane neuronal 4 (LRRTM4) gene. Although no correlations between this gene and *H. pylori* have been reported, a previous study described that the extracellular leucine rich repeat proteins may play a role in neural development, innate immunity and inflammation.[20] Our results could therefore provide novel insights in the *H. pylori* susceptibility and basic research is necessary to confirm our genome wide association and provide functional evidence supporting the hypothesis that the LRRTM4 gene, the LOH11CR2A gene or the olfactory receptor genes are to some extent involved in *H. pylori* susceptibility.

Although this study provides suggestive genome wide evidence for an association between two loci and *H. pylori* susceptibility, potential limitations of this study warrant consideration.

Firstly, to provide consistent genome wide evidence, larger populations of cases and controls and combination of genome wide results of similar studies are necessary. Nevertheless, given the paucity of literature on genetic polymorphisms regulating *H. pylori* susceptibility and the difficulties to collect DNA and provide genome wide evidence, our study is a first step towards clarification of *H. pylori* susceptibility. Secondly, significant differences existed between our discovery and replication cohort. In particular, gender differences were considerable as the discovery cohort consisted of only women. However, since a previous Dutch study demonstrated that the prevalence of *H. pylori* was not significantly different between male and female healthy blood donors and since our results do not correlate *H. pylori* status with gender-related genetic factors, we are confident that the results found in the female discovery cohort can also applied to males and the difference between these cohorts are therefore justified.[21]

In conclusion, although the identity of the underlying loci of the Genome-Wide Associated SNPs remains elusive and the function of the nearest genes is obscure, our study provides compelling evidence for the existence of at least one genetic region (rs17015126 on chromosome 2) that may play a role in *H. pylori* susceptibility.

## REFERENCES


1 Parsonnet J. Helicobacter pylori: the size of the problem. *Gut* 1998;43 Suppl 1:S6-9.
2 Amieva MR, El-Omar EM. Host-bacterial interactions in Helicobacter pylori infection. *Gastroenterology* 2008;134:306-23.
3 Suerbaum S, Michetti P. Helicobacter pylori infection. *N Engl J Med* 2002;347:1175-86.
4 Go MF. Review article: natural history and epidemiology of Helicobacter pylori infection. *Aliment Pharmacol Ther* 2002;16 Suppl 1:3-15.
5 Farinha P, Gascoyne RD. Helicobacter pylori and MALT lymphoma. *Gastroenterology* 2005;128: 1579-605.
6 Perez-Perez GI, Rothenbacher D, Brenner H. Epidemiology of Helicobacter pylori infection. *Helicobacter* 2004;9 Suppl 1:1-6.
7 Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of Helicobacter pylori infection. *Clin Microbiol Rev* 2006;19:449-90.
8 Holcombe C, Omotara BA, Eldridge J*, et al.* H. pylori, the most common bacterial infection in Africa: a random serological study. *Am J Gastroenterol* 1992;87:28-30.
9 Segal I, Ally R, Mitchell H. Helicobacter pylori--an African perspective. *Qjm* 2001;94:561-5.
10 Bardhan PK. Epidemiological features of Helicobacter pylori infection in developing countries. *Clin Infect Dis* 1997;25:973-8.
11 Malaty HM, Engstrand L, Pedersen NL*, et al.* Helicobacter pylori infection: genetic and environmental influences. A study of twins. *Ann Intern Med* 1994;120:982-6.
12 Hofman A, Breteler MM, van Duijn CM*, et al.* The Rotterdam Study: 2010 objectives and design update. *Eur J Epidemiol* 2009;24:553-72.
13 Purcell S, Neale B, Todd-Brown K*, et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;81:559-75.
14 Liou JM, Lin JT, Wang HP*, et al.* IL-1B-511 C-->T polymorphism is associated with increased host susceptibility to Helicobacter pylori infection in Chinese. *Helicobacter* 2007;12:142-9.
15 El-Omar EM, Rabkin CS, Gammon MD*, et al.* Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. *Gastroenterology* 2003;124:1193-201.
16 El-Omar EM, Carrington M, Chow WH*, et al.* Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000;404:398-402.
17 Tseng FC, Brown EE, Maiese EM*, et al.* Polymorphisms in cytokine genes and risk of Helicobacter pylori infection among Jamaican children. *Helicobacter* 2006;11:425-30.
18 Martin ES, Cesari R, Pentimalli F*, et al.* The BCSC-1 locus at chromosome 11q23-q24 is a candidate tumor suppressor gene. *Proc Natl Acad Sci U S A* 2003;100:11517-22.
19 Olender T, Lancet D, Nebert DW. Update on the olfactory receptor (OR) gene superfamily. *Hum Genomics* 2008;3:87-97.
20 Dolan J, Walshe K, Alsbury S*, et al.* The extracellular leucine-rich repeat superfamily; a comparative survey and analysis of evolutionary relationships and expression patterns. *BMC Genomics* 2007;8:320.
21 Loffeld RJ, Stobberingh E, van Spreeuwel JP*, et al.* The prevalence of anti-Helicobacter (Campylobacter) pylori antibodies in patients and healthy blood donors. *J Med Microbiol* 1990;32:105-9.

# Chapter 3

## Gastric MALT lymphoma: Epidemiology and high adenocarcinoma risk in a nation-wide study

Lisette G. Capelle[1], Annemarie C. de Vries[1], Caspar W. N. Looman[2], Mariel K. Casparie[3], Henk Boot[4], Gerrit A. Meijer[5] and Ernst J. Kuipers[1]

[1]Departments of Gastroenterology and Hepatology, and [2]Public Health, Erasmus MC University Medical Center, Rotterdam, [3]Stichting PALGA, The Netherlands, [4]Department of Gastroenterology, Antoni van Leeuwenhoek Hospital, Netherlands Cancer Institute, and [5]Department of Pathology, Free University Medical Center, Amsterdam

*European Journal of Cancer 2008;44(16):2470-6*

## ABSTRACT

*Background:* Gastric marginal zone non-Hodgkin lymphomas MALT type (gMALT) and gastric adenocarcinomas (GC) are long-term complications of chronic *Helicobacter pylori* gastritis, however, the incidence of gMALT and the GC risk in these patients is unclear.
*Objective:* To evaluate epidemiological time trends of gMALT in the Netherlands and to estimate GC risk.
*Methods:* Patients with a first diagnosis of gMALT between 1991 and 2006 were identified in the Dutch nation-wide histopathology registry (PALGA). Age-standardised incidence rates were calculated. The incidences of GC in patients with gMALT and in the Dutch population were compared. Relative risks were calculated by a Poisson Model.
*Results*: In total, 1419 patients were newly diagnosed with gMALT, compatible with an incidence of 0.41/100,000/year. GC was diagnosed in 34 (2.4%) patients of the cohort. Patients with gMALT had a sixfold increased risk for GC in comparison with the general population ($p < 0.001$). This risk was 16.6 times higher in gMALT patients aged between 45 and 59 years than in the Dutch population ($p < 0.001$).
*Conclusions:* GC risk in patients with gMALT is six times higher than in the Dutch population and warrants accurate re-evaluation after diagnosis and treatment for gMALT.

## INTRODUCTION

*Helicobacter pylori* causes chronic inflammation of the gastric mucosa in virtually all infected subjects. This inflammatory process can progress through the pre-malignant stages of atrophic gastritis, intestinal metaplasia and dysplasia to gastric adenocarcinomas.[1,2] As such, *H. pylori* infection is the most important risk factor for the development of gastric adenocarcinomas. Although, the incidence of gastric cancer is declining in the Western world, gastric cancer remains the 4th most common cancer and second leading cause of cancer-related death worldwide.[3,4] The declining incidence of gastric cancer in Western countries is similar to the declining incidence of peptic ulcer disease, attributed to the declining *H. pylori* prevalence.[5,6]

In addition, *H. pylori* infection has increasingly been recognised in the pathogenesis of gastric mucosa-associated lymphoid tissue lymphomas (gMALT).[7,8] Although gMALTs are also strongly associated with *H. pylori* infection, the incidence of this condition has, in contrast to the gastric cancer incidence, been reported to increase.[8-12] It is controversial whether this is a true increase with a shift in outcomes of *H. pylori* infection. Alternatively, changes in the number of endoscopic procedures, biopsy sampling protocols and histological criteria could have influenced the number of diagnoses.[12] Progression of low-grade gMALT is slow, and *H. pylori* eradication alone leads to partial or complete remission in 60–80% of patients, in particular those without a specific *API2-MALT1 t*(11;18) chromosomal translocation.[2,13] On the contrary, gastric cancer is usually diagnosed at an advanced stage with only limited curative options and consequently a low 5-year survival rate. Although both conditions are long-term complications of chronic *H. pylori* infection, the potential interrelation is unclear and it is controversial whether gastric cancer risk is increased in patients with gMALT. Previous case series and small cohort studies described the occurrence of adenocarcinomas simultaneously or during follow-up of gMALT,[14-18] however, other studies could not confirm these observations.[11,19-21] In addition, a recent study observed increased progression of pre-malignant gastric lesions in patients with gMALT as compared to patients with non-complicated gastritis.[13] On the basis of these contrasting data and in the absence of long-term data in larger cohorts, the risk for gastric cancer in patients with gMALT remains unclear.

Therefore, the aim of this study was to evaluate epidemiological time trends of gMALT in the Netherlands and to evaluate gastric cancer risk in patients with a diagnosis of gMALT.

## METHODS

### *Histopathology database*

In the Netherlands, all histopathology and cytopathology reports are collected in a national archive (PALGA database), which has nation-wide coverage since 1991.[22] Patients in this database are identified by date of birth, gender and the first four characters of their family name. Though sometimes identities of two patients are falsely matched, this identification string enables the linkage of different tests belonging to the same patient, and therefore also to follow individual testing histories (dates and diagnoses) irrespective of the facility of treatment.[23]

All specimens receive a diagnostic code, similar to the Systematised Nomenclature of Medicine (SNOMED) classification of the College of American Pathologists.[24] This code consists of a term indicating the anatomical location, type of sample and a morphological term describing the finding. The records in the database contain these codes and the summary of the pathology report. In this study, data recorded in the PALGA database between 1991 and 2006 were included. For each report, gender, date of birth, date of pathology report, summary text and diagnostic codes were made available.

### *Patient selection*

All patients with a histologically confirmed diagnosis of gMALT were identified in the database. The diagnostic codes that were used to identify the patients with gMALT are described in Appendix. To evaluate the incidence of gMALT in different age classes, incidence numbers in different periods were calculated within the 5-year age groups. The ratio of the number of new patients with a positive biopsy for gMALT to the number of new patients with a first time gastric biopsy was calculated, in order to correct for possible changes in frequency of upper gastro-intestinal endoscopies with biopsy sampling.

Within the cohort of patients with a gMALT, all patients with a histologically confirmed diagnosis of gastric cancer were identified. Timing of gastric cancer diagnosis was evaluated with regard to diagnosis of gMALT. In this evaluation, patients with a gastric cancer diagnosis simultaneously with, or within one year prior to or after diagnosis of gMALT were considered concomitant diagnoses.

In addition, all patients with a diagnosis of atrophic gastritis, intestinal metaplasia or dysplasia prior to, simultaneous with, or after the diagnosis of gMALT were identified.

### *Statistical analysis*

Age-standardised incidence rates (World standardised rate, WSR) of histologically confirmed gMALT were evaluated for the study period. To compare categorical and continuous variables between patients with low, intermediate to high and undefined grade gMALT, $\chi^2$-tests, *t*-tests and one way ANOVA tests were used, considering a two-sided *p*-value <0.05 as statistically significant.

To calculate the relative risk of gastric cancer in patients with gMALT, the incidence of gastric cancer observed in patients with gMALT was compared to the incidence of gastric cancers in the general Dutch population from 1991 to 2006 and aggregated over age and sex. As the PALGA registry does not contain date of death of patients, unless an autopsy had been performed, the person-years at risk would be overestimated. Therefore, we imputed death to get a correct estimate of the number of person-years at risk for all patients that did not develop gastric cancer during follow-up. Starting from the calendar year, age and gender of the persons, we collected the survival data from the general Dutch population for ever open-ended follow-up. Drawing from a binomial distribution for every year then yielded a dataset with an approximately unbiased number of years-at-risk. The number of patients is large, but we tried multiple imputation, that did not change the results, as was to be expected. The incidence of gastric cancer in the Dutch population was calculated on the basis of the total number of gastric cancers registered in the PALGA database and the midyear Dutch population.[25] A Poisson Model, corrected for age categories, gender and calendar year, was used for calculating the relative risks and 95% confidence intervals (CIs).



## RESULTS

Between 1991 and 2006, 1419 patients were newly diagnosed with gMALT, 972 patients were initially diagnosed with a low-grade lymphoma, 357 patients with an intermediate to high-grade lymphoma and in 90 patients the grade of the lymphoma was undefined (Table 1). Within the group of patients with a low-grade lymphoma, 32 (3.3%) patients developed a high-grade lymphoma within 1 to 8 years.

**Table 1.** Baseline characteristics

| | Total | Low grade | Intermediate to high grade | Undefined grade |
|---|---|---|---|---|
| **Number of patients with gastric MALT lymphoma** | 1419 | 972 (68.5%) | 357 (25.2%) | 90(6.3%) |
| **Male/Female (%)** | 51.9/48.1 | 51.3/48.7 | 53.5/46.5 | 52.2/47.8 |
| **Age** | | | | |
| Median (yrs) | 68.0 | 67.0 | 70.6 | 68.7 |
| Percentile 25$^{th}$ and 75$^{th}$ | 57.6/76.7 | 57.1/75.4 | 58.9/78.7 | 57.1/76.2 |

### *Epidemiology*

Overall, the mean age of patients at diagnosis of gMALT was 66.1 (SD 14.1) years (range 13.7–98.2 years), and the peak incidence of gMALT both in men and women was between 70 and 74 years (Fig. 1). The proportion of male to female patients in the cohort was 51.9 to 48.1% (Table 1). No significant differences in male to female ratios were observed between patients with low-grade, intermediate to high-grade or undefined grade gMALT ($p = 0.78$). Patients with an initial diagnosis of low-grade gMALT (median age 67.0 years) were significantly younger compared to patients with intermediate to high-grade gMALT (median age 70.6 years) ($p = 0.002$). Age at diagnosis was significantly higher in females as compared to males, both in patients with low-grade gMALT ($p = 0.03$) and intermediate to high-grade gMALT ($p = 0.001$).

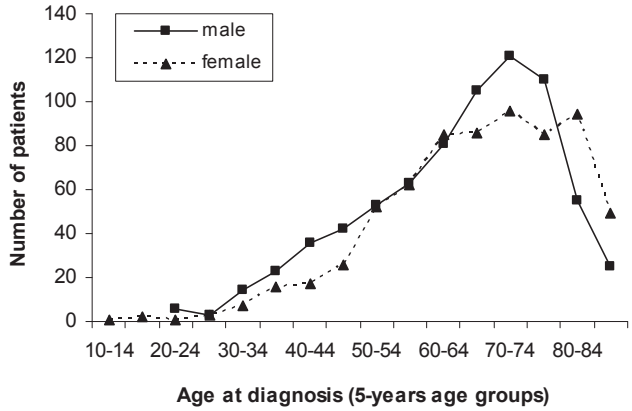


**Figure 1.** Age at gastric MALT lymphoma diagnosis

Over the whole study period, the average number of new diagnoses of gMALT was 88.7 cases per year, and the age standardised incidence rate was 0.41 per 100,000 per year (WSR) (Fig. 2). This incidence was not stable over the total study period. At first, the incidence of gMALT increased with 5.8% (95% CI 1.9–9.9%) per year in the period from 1991 to 1997. This was followed by an annual 8.8% (95% CI 6.2–11.4%) decline until 2006 (Fig. 2). Altogether, this corresponded with an annual WSR of 0.28 per 100,000 in 1991, increasing to a maximum of 0.72 in 1997, followed by a decrease to 0.27 in 2006. Gastric MALT lymphoma was diagnosed significantly more often in the period from 1991 to 2000 as compared to the period from 2001 to 2006 ($p < 0.001$).

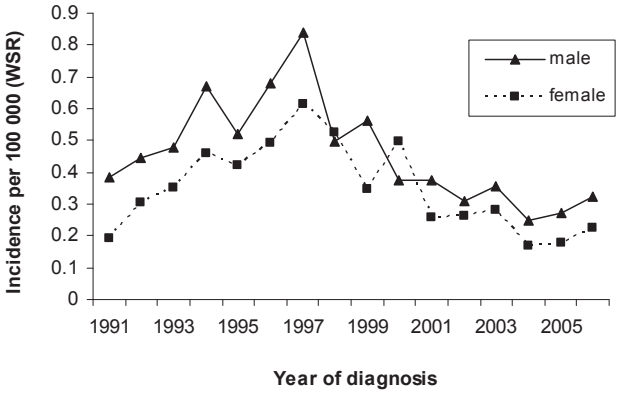


**Figure 2.** The incidence of gastric MALT lymphoma (WSR, World Standardised Rate) in the Netherlands



## *Gastric cancer risk*

In total, 34 (2.4%) gMALT patients (18 males, 16 females) were diagnosed with gastric cancer at a median age of 72.0 years (SD 9.6). This comprised 2.7% of 1244 patients in whom no gastrectomy was performed after diagnosis of gMALT. Gastric cancer was diagnosed prior to the diagnosis of gMALT in 3 (8.8%) patients, in 18 (52.9%) patients both malignancies were diagnosed simultaneously (i.e. within a time frame of one year), and in 13 (38.2%) patients gastric cancer was diagnosed more than one year after the gMALT diagnosis (Table 2). The median interval between gastric cancer and gMALT in patients with gastric cancer development after diagnosis of gMALT was 6.0 (range 1.1–7.4) years.

Details on stage of gastric cancer were provided in 15 (44%) patients. Five (15%) patients were diagnosed at a stage of early gastric cancer, however, in 10 (29.4%) patients the tumour was already invading the lamina propria, submucosa or beyond. In addition, lymph nodes

**Table 2.** Gastric MALT lymphoma and gastric cancer diagnosis

| | Total | Low grade | Intermediate to high grade | Undefined grade |
|---|---|---|---|---|
| **Timing of gastric cancer diagnosis** | | | | |
| Prior to MALT lymphoma (%) | 3(8.8) | 3(10.7) | 0 | 0 |
| Concomitant with MALT lymphoma (%) | 18(52.9) | 16(57.1) | 1(20.0) | 1(100) |
| After MALT lymphoma (%) | 13(38.2) | 9(32.1) | 4(80.0) | 0 |
| **Male/Female (%)** | 52.9/47.1 | 60.7/39.3 | 20.0/80.0 | 0/100 |
| **Age** | | | | |
| Median (yrs) | 72.0 | 73.2 | 70.2 | 72.2 |
| Percentile 25$^{th}$ and 75$^{th}$ | 65.5/78.7 | 64.2/78.2 | 61.0/86.9 | |

were involved in 4 (11.8%) patients, as demonstrated by histological evaluation after gastric resection.

Overall, the study population contained 440 (31%) patients with a diagnosis of a pre-malignant gastric lesion prior to, simultaneously with, or after the diagnosis of gMALT, of which 65 (4.6%) patients were diagnosed with atrophic gastritis, 302 (21.3%) patients with intestinal metaplasia and 73 (5.1%) patients with dysplasia. In 21% of these patients a diagnosis of atrophic gastritis, intestinal metaplasia or dysplasia preceded the diagnosis of gastric cancer.

Gastric cancer risk was not significantly different between patients with low, intermediate to high or undefined grade gMALT ($p = 0.21$). In addition, no significant differences in gastric cancer risk were demonstrated between male and female patients ($p = 0.91$).

Overall, patients with a diagnosis of gMALT were at a six times higher risk of developing gastric cancer as compared to the general Dutch population (Table 3). Males with gMALT had a 4.4 times higher risk as compared to the general population ($p < 0.001$), whereas females had a 10.0 times higher risk ($p < 0.001$). The relative risk of gastric cancer was significantly higher in female patients with a gMALT as compared to male patients ($p = 0.02$). However, the absolute risk of gastric cancer for males and females older than 45 years was not significantly different (respectively, 4.0/1000 person-years and 4.3/1000 person-years; $p = 0.81$). Gastric cancer risk was 16.6 times increased in patients aged between 45 and 59 years as compared to the general Dutch population ($p < 0.001$), 10-fold increased in patients aged between 60 years and 74 years and threefold increased in those above 74 years (Table 3). These differences in relative risk for the age groups were significant ($p = 0.004$). However, the absolute gastric cancer risk in patients with gMALT did not differ between those aged 45 to 59 years and those above 59 years ($p = 0.07$).

**Table 3.** The relative risk of gastric cancer (GC) in patients with gastric MALT lymphoma (gMALT) as compared to the general Dutch population.

| | | GC in Dutch population | GC in gMALT patients | Relative risk | 95%CI | P value for difference |
|---|---|---|---|---|---|---|
| **Overall** | | 36577 | 30 | 6.11 | [4.28-8.72] | |
| **Sex** | Male | 22778 | 15 | 4.39 | [2.65-7.28] | 0.02 |
| | Female | 13799 | 15 | 10.04 | [6.07-16.60] | |
| **Age at baseline** | 45-59 yrs | 6229 | 5 | 16.64 | [5.45-50.80] | 0.004 |
| | 60-74 yrs | 15253 | 17 | 10.64 | [6.52-17.4] | |
| | ≥ 75 yrs | 13666 | 8 | 3.43 | [1.91-6.13] | |

## DISCUSSION

First of all this study provides long-term nation-wide data on the incidence of gMALT in a Western population. It shows an overall incidence of gMALT of approximately 0.4/100,000/year. Secondly, our data show that this incidence has considerably changed over the past

18 years, initially increasing between 1991 and 1997, which was followed by a rapid decline. Thirdly, we provide long-term data that confirm the suggestion from previous case reports that gMALT patients have a considerably higher gastric cancer risk than the general population. In most cases, gastric cancer is diagnosed within one year prior to or after the diagnosis of gMALT. Therefore, on the basis of our data, accurate evaluation of gMALT seems to be warranted for a diagnosis of gastric cancer concomitantly or after the diagnosis of gMALT.

Our data demonstrate that gMALT is a relatively rare disease in a Western population. Previous studies in Western countries have demonstrated incidences varying between 0.21/100,000 (England) and 13/100,000 (Italy).[2,26,27] These differences are probably explained by differences in the prevalence of *H. pylori* between the studied populations, study power based on the magnitude of the study population, the period of follow-up and the timing of the study.[2,26,28] In our population, a diagnosis of gMALT was not extremely rare as approximately 0.2% of the total number of patients with a first gastric biopsy over the study period were diagnosed with a gMALT.

Previous studies described an increasing incidence of gastric lymphomas in contrast to the declining incidences of *H. pylori* infection, peptic ulcer disease, atrophic gastritis, intestinal metaplasia and gastric adenocarcinomas.[5,6,12] Our data similarly demonstrate that the incidence of gMALT increased from 1991 to 1997, but decreased rather rapidly thereafter. The initial increase is probably related to the increasing interest in this diagnosis after the discovery of an association between *H. pylori* infection and gMALT in 1991.[8] The importance of *H. pylori* as risk factor for MALT lymphoma was confirmed by the regression of low-grade MALT lymphoma after *H. pylori* eradication.[19,29] Thereby, gMALT became an infection-associated malignant disease.[2] This led in a change of primary treatment strategy from chemoradiotherapy and surgery to *H. pylori* eradication therapy. This major change may have contributed to an increase in the number of new cases diagnosed with gMALT during those years. Furthermore, improved endoscopic and histological diagnostic procedures may also have contributed to the increasing incidence of gMALTs.[30-32] For several years, all non-Hodgkin lymphomas (NHLs) were classified following the Working Formulation (WF) in low-grade and high-grade lymphomas. This working formulation did not include several morphologic and clinical distinct entities, including gMALT. Consensus for a more multifaceted approach to NHLs was reached in a revised European–American lymphoma (REAL) classification in 1993, which recognised the mucosa-associated lymphomas.[33] Thereafter, gMALTs were considered a specific entity.[2] Currently their incidence is rapidly declining. This decline is likely in part related to the current decline in the prevalence of *H. pylori* in Western countries. However, the decline of incidence of gMALT is much more rapid than the declining *H. pylori* prevalence.[5,34,35] Therefore, other factors must additionally play a role and need to be further investigated.

Although several case series were published on synchronous and metachronous occurrence of both gastric cancer and gMALT, it remained unclear whether gastric cancer risk was increased in gMALT patients compared to the general population.[11,14,16,19,36-38] Our study dem-

onstrates this risk is indeed about six times increased (Table 3). The absolute risk was equal in male and female gMALT patients, which contrasts with the general population, where the risk for gastric cancer is considerably higher in men. Thus, the relative risk of gastric cancer in MALT patients is higher in women than in men. Similarly, the gastric cancer risk was the same in younger and elderly gMALT patients, and thus the relative risk for gastric cancer was significantly higher in younger MALT lymphoma patients (Table 3). The relative risks of gastric cancer after a diagnosis of gMALT described in our study could even be higher since gastrectomy was performed in 175 patients after diagnosis of a gMALT, in particular in the early years when *H. pylori* eradication was not yet an accepted treatment method.

As patients with gastric MALT lymphoma are already at an increased risk of developing gastric cancer by being *H. pylori* positive, a further comparison between *H. pylori*-positive subgroups is essential. Previous studies demonstrated that *H. pylori* infection increased gastric cancer risk at least twofold resulting for *H. pylori*-positives in an estimated lifetime risk for gastric cancer of approximately 1%.[39,40] In addition, we recently published a study describing the risk of gastric cancer in a large cohort of patients with atrophic gastritis and intestinal metaplasia, which occurs like MALT lymphoma against a background of *H. pylori* infection. This study demonstrated that within ten years of follow-up the gastric cancer risk in these subjects with a pre-neoplastic condition varied between the two and three percent.[41] This background supports the conclusion that patients with gMALT are at increased risk for gastric cancer compared to *H. pylori*-positive subjects, and that this risk is in fact very similar to patients with atrophic gastritis and intestinal metaplasia.[41]

In 38% of patients with diagnosis of gastric cancer, gastric cancer was diagnosed after gMALT with a median interval of 6.0 years (range1–7). This interval is similar to the interval observed in a review of previous cases on metachronous occurrence of gMALT which reported 6 months to 5 years.[16] However, the exact period between diagnosis of a gMALT and cancer or remission is difficult to interpret, since different histological scoring systems have been used to evaluate lymphoma response to therapy over the past decade.[29,42] As these grading systems demonstrated low interobserver reproducibility, a new grading system based on evaluation of diagnostic features of lymphoepithelial changes was put forward.[43] According to this grading system, a recent study described a favourable disease course of patients treated with *H. pylori* eradication, after 42.2 months of follow-up, in which one-third of the patients went into complete remission.[21,43] However, the findings in our study emphasise the need of accurate endoscopic and histological re-evaluation of the gastric mucosa after diagnosis of a gMALT, since the majority who developed gastric cancer was diagnosed with adenocarcinoma concomitantly (52.9%) with their gMALT or during later surveillance (38.2%).

Although this study describes a large nation-wide cohort of patients with gMALT with long-term follow-up, potential limitations of this study warrant consideration.

Firstly, for most of the period under study, MALT lymphomas were classified as either low- or high-grade and it is therefore that our report included cases under these search terms. At present, gMALTs are considered as a specific disease entity of marginal zone lymphoma (mucosa-associated lymphoid tissue lymphoma (MALT) type), which led to the formalised WHO classification, according to which these lesions are now referred to as gastric marginal zone lymphomas MALT type.[44] Also, the term high-grade MALT lymphoma was replaced by Diffuse Large B-Cell Lymphoma (DLBCL) in this new classification, as it was discovered that low-grade and high-grade gMALTs have a different histogenesis.[44] These DLBCLs may contain a low-grade MALT lymphoma component. However, it remains unclear to which extent they transformed from low-grade MALT lymphomas *versus de novo* DLBCLs.[45] For these reasons, it is likely that a small proportion of the high-grade gastric MALT lymphomas in our cohort included DLBCLs unrelated to MALT. However, these changes of nomenclature have not led to a major change in diagnoses and therefore unlikely affected the main outcome parameters of our study, i.e. the incidence of gMALTs and the risk for gastric cancer in these patients. Secondly, we could not evaluate the extension of pre-malignant gastric lesions in the mucosa surrounding the MALT lymphomas, as the relatively low percentage of patients with gastric atrophy, intestinal metaplasia and dysplasia prior to or simultaneous with gMALT diagnosis made this impossible. In addition, details on location and invasion of the MALT lymphomas were not provided. Lymphomas tend to occur proximally in the stomach, whereas gastric adenocarcinomas occur more distal.[36] For these reasons, details on extension of pre-malignant gastric lesion, and size and depth of MALT lymphoma might identify patients at higher risk and consequently lead to more accurate surveillance. Similarly, evaluating the gastric cancer risk in the cohort after stratification by *H. pylori* and translocation status may also result in more accurate surveillance and prognosis. Previous studies observed the specific *API2-MALT1 t*(11;18) chromosomal translocation in approximately 30% (range 18–40%) of gMALT patients.[2,46,47] Most patients with this specific translocation do not respond to *H. pylori* eradication and demonstrate dissemination to regional lymph nodes or distal sites than the stomach more frequently. Development of gastric cancer was reported to occur in translocation-positive patients. However, these case series were very small and the exact risk of developing gastric cancer remained unclear.[48,49] For these reasons, a large prospective study of patients with gMALT and determination of their translocation status is essential to evaluate patients at high risk of developing gastric cancer, however, the rare appearance of gMALTs will make this study hardly feasible. Thirdly, as limited numbers of biopsies can provide insufficient information for subtyping, and determination of horizontal extension and multifocality of gMALTs, previous studies described the need for a standardised protocol taking 20–30 biopsies from involved and uninvolved mucosa both at baseline and during follow-up.[32,50] However, we could not evaluate the number and distribution of biopsies obtained within each individual case and at every time point. Therefore, the number of patients with in particular pre-malignant gastric lesions after a diagnosis of gMALT may have been overdi-

agnosed.[51] Finally, a previous study proposed that gMALT patients treated with chemo- and/or radiotherapy were particularly at increased risk for gastric cancer,[52] but we were unable to assess this in our study population as we lack details with respect to chemoradiotherapy that without doubt has been given to patients during the first years of our study period.

In conclusion, the overall incidence of gMALT is low and currently declining, which is likely related to the current decline in the prevalence of *H. pylori* infections, but also has to be due to other unidentified factors as the decline is considerably more rapid than the decline of *H. pylori* prevalence. After a diagnosis of gMALT, an accurate endoscopic and histological re-evaluation of the gastric mucosa seem to be warranted as gastric cancer risk in patients with gMALT is substantial and the majority who develop gastric cancer are diagnosed concomitantly or after their gMALT. Future research is needed to clarify the clinical course of these patients in order to improve treatment and prognosis of patients with gMALT.

## ACKNOWLEDGEMENT

This study was made possible by an unrestricted grant from Nycomed BV, Hoofddorp, The Netherlands.

## APPENDIX A

The following SNOMED-like codes were used:

- *Stomach*: T63000.
- *Atrophic gastritis*: M58000, M58001, M58010.
- *Intestinal metaplasia*: M73000, M73200, M73320, M73321, M73300.
- *Dysplasia*: M74000, M74006, M74007, M74008, M74009.
- *Gastric cancer*: M81403, M80103, M84803, M81443, M81453, M84903, M82113, M80503, M82603, M69360, M81404, M80104, M80105, M80123, M80193, M80213, M80203.
- *MALT lymphoma*: M97153, M97183, M97163, M96993, M97183.
- *Malignant lymphoma/malignant non-Hodgkin lymfoma*: M95903, F40640.

## REFERENCES


1 Correa P. Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 1992;52:6735-40.

2 Farinha P, Gascoyne RD. Helicobacter pylori and MALT lymphoma. *Gastroenterology* 2005;128: 1579-605.

3 Fox JG, Wang TC. Inflammation, atrophy, and gastric cancer. *J Clin Invest* 2007;117:60-9.

4 Ferlay J BF, Pisani P, *et al.* . *GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide. IARC CancerBase No 5 version 2.0*. Lyon: IARCPress. 2004.

5 de Vries AC, Meijer GA, Looman CW, *et al.* Epidemiological trends of pre-malignant gastric lesions: a long-term nationwide study in the Netherlands. *Gut* 2007;56:1665-70.

6 Post PN, Kuipers EJ, Meijer GA. Declining incidence of peptic ulcer but not of its complications: a nation-wide study in The Netherlands. *Aliment Pharmacol Ther* 2006;23:1587-93.

7 Isaacson P, Wright DH. Malignant lymphoma of mucosa-associated lymphoid tissue. A distinctive type of B-cell lymphoma. *Cancer* 1983;52:1410-6.

8 Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, *et al.* Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. *Lancet* 1991;338:1175-6.

9 Gurney KA, Cartwright RA, Gilman EA. Descriptive epidemiology of gastrointestinal non-Hodgkin's lymphoma in a population-based registry. *Br J Cancer* 1999;79:1929-34.

10 Stolte M, Bayerdorffer E, Morgner A, *et al.* Helicobacter and gastric MALT lymphoma. *Gut* 2002;50 Suppl 3:III19-24.

11 Bayerdorffer E, Miehlke S, Neubauer A, *et al.* Gastric MALT-lymphoma and Helicobacter pylori infection. *Aliment Pharmacol Ther* 1997;11 Suppl 1:89-94.

12 Severson RK, Davis S. Increasing incidence of primary gastric lymphoma. *Cancer* 1990;66:1283-7.

13 Lamarque D, Levy M, Chaumette MT, *et al.* Frequent and rapid progression of atrophy and intestinal metaplasia in gastric mucosa of patients with MALT lymphoma. *Am J Gastroenterol* 2006;101: 1886-93.

14 Wundisch T, Thiede C, Morgner A, *et al.* Long-term follow-up of gastric MALT lymphoma after Helicobacter pylori eradication. *J Clin Oncol* 2005;23:8018-24.

15 Morgner A, Miehlke S, Stolte M, *et al.* Development of early gastric cancer 4 and 5 years after complete remission of Helicobacter pylori associated gastric low grade marginal zone B cell lymphoma of MALT type. *World J Gastroenterol* 2001;7:248-53.

16 Hamaloglu E, Topaloglu S, Ozdemir A, *et al.* Synchronous and metachronous occurrence of gastric adenocarcinoma and gastric lymphoma: A review of the literature. *World J Gastroenterol* 2006;12: 3564-74.

17 Goteri G, Ranaldi R, Rezai B, *et al.* Synchronous mucosa-associated lymphoid tissue lymphoma and adenocarcinoma of the stomach. *Am J Surg Pathol* 1997;21:505-9.

18 Arista-Nasr J, Jimenez-Rosas F, Uribe-Uribe N, *et al.* Pathological disorders of the gastric mucosa surrounding carcinomas and primary lymphomas. *Am J Gastroenterol* 2001;96:1746-50.

19 Bayerdorffer E, Neubauer A, Rudolph B, *et al.* Regression of primary gastric lymphoma of mucosa-associated lymphoid tissue type after cure of Helicobacter pylori infection. MALT Lymphoma Study Group. *Lancet* 1995;345:1591-4.

20 Au WY, Gascoyne RD, Le N, *et al.* Incidence of second neoplasms in patients with MALT lymphoma: no increase in risk above the background population. *Ann Oncol* 1999;10:317-21.

21 Fischbach W, Goebeler ME, Ruskone-Fourmestraux A, *et al.* Most patients with minimal histological residuals of gastric MALT lymphoma after successful eradication of Helicobacter pylori can be

managed safely by a watch and wait strategy: experience from a large international series. *Gut* 2007;56:1685-7.

22 Casparie M, Tiebosch AT, Burger G, *et al.* Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. *Cell Oncol* 2007;29:19-24.

23 Van den Brandt PA, Schouten LJ, Goldbohm RA, *et al.* Development of a record linkage protocol for use in the Dutch Cancer Registry for Epidemiological Research. *Int J Epidemiol* 1990;19:553-8.

24 Cote RA, Robboy S. Progress in medical information management. Systematized nomenclature of medicine (SNOMED). *Jama* 1980;243:756-62.

25 www.cbs.nl. Statistics Netherlands. 2007.

26 Doglioni C, Wotherspoon AC, Moschini A, *et al.* High incidence of primary gastric lymphoma in northeastern Italy. *Lancet* 1992;339:834-5.

27 Ullrich A, Fischbach W, Blettner M. Incidence of gastric B-cell lymphomas: a population-based study in Germany. *Ann Oncol* 2002;13:1120-7.

28 Loffeld RJ, van der Putten AB. Changes in prevalence of Helicobacter pylori infection in two groups of patients undergoing endoscopy and living in the same region in the Netherlands. *Scand J Gastroenterol* 2003;38:938-41.

29 Wotherspoon AC, Doglioni C, Diss TC, *et al.* Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of Helicobacter pylori. *Lancet* 1993;342:575-7.

30 Brands F, Monig SP, Raab M. Treatment and prognosis of gastric lymphoma. *Eur J Surg* 1997;163: 803-13.

31 Koch P, Probst A, Berdel WE, *et al.* Treatment results in localized primary gastric lymphoma: data of patients registered within the German multicenter study (GIT NHL 02/96). *J Clin Oncol* 2005;23: 7050-9.

32 Fischbach W, Dragosics B, Kolve-Goebeler ME, *et al.* Primary gastric B-cell lymphoma: results of a prospective multicenter study. The German-Austrian Gastrointestinal Lymphoma Study Group. *Gastroenterology* 2000;119:1191-202.

33 Harris NL, Jaffe ES, Stein H, *et al.* A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994;84:1361-92.

34 Loffeld RJ, Stobberingh E, van Spreeuwel JP, *et al.* The prevalence of anti-Helicobacter (Campylobacter) pylori antibodies in patients and healthy blood donors. *J Med Microbiol* 1990;32:105-9.

35 van Vuuren AJ dMR, van Driel HF, et al. Seroprevalence of Helicobacter pylori in two asymptomatic Dutch populations. *Gastroenterology* 2006;130(Suppl 2):T1895.

36 Nakamura S, Aoyagi K, Iwanaga S, *et al.* Synchronous and metachronous primary gastric lymphoma and adenocarcinoma: a clinicopathological study of 12 patients. *Cancer* 1997;79:1077-85.

37 Chan AO, Chu KM, Yuen ST, *et al.* Synchronous gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma in association with Helicobacter pylori infection: comparing reported cases between the East and West. *Am J Gastroenterol* 2001;96:1922-4.

38 Zucca E, Pinotti G, Roggero E, *et al.* High incidence of other neoplasms in patients with low-grade gastric MALT lymphoma. *Ann Oncol* 1995;6:726-8.

39 Huang JQ, Zheng GF, Sumanac K, *et al.* Meta-analysis of the relationship between cagA seropositivity and gastric cancer. *Gastroenterology* 2003;125:1636-44.

40 Kuipers EJ. Review article: exploring the link between Helicobacter pylori and gastric cancer. *Aliment Pharmacol Ther* 1999;13 Suppl 1:3-11.

41 de Vries AC, van Grieken NC, Looman CW, *et al.* Gastric cancer risk in patients with premalignant gastric lesions: a nationwide cohort study in the Netherlands. *Gastroenterology* 2008;134:945-52.
42 Neubauer A, Thiede C, Morgner A, *et al.* Cure of Helicobacter pylori infection and duration of remission of low-grade gastric mucosa-associated lymphoid tissue lymphoma. *J Natl Cancer Inst* 1997;89:1350-5.
43 Copie-Bergman C, Gaulard P, Lavergne-Slove A, *et al.* Proposal for a new histological grading system for post-treatment evaluation of gastric MALT lymphoma. *Gut* 2003;52:1656.
44 Harris NL, Jaffe ES, Diebold J, *et al.* World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting-Airlie House, Virginia, November 1997. *J Clin Oncol* 1999;17:3835-49.
45 Du MQ, Atherton JC. Molecular subtyping of gastric MALT lymphomas: implications for prognosis and management. *Gut* 2006;55:886-93.
46 Ye H, Liu H, Attygalle A, *et al.* Variable frequencies of t(11;18)(q21;q21) in MALT lymphomas of different sites: significant association with CagA strains of H pylori in gastric MALT lymphoma. *Blood* 2003;102:1012-8.
47 Inagaki H, Nakamura T, Li C, *et al.* Gastric MALT lymphomas are divided into three groups based on responsiveness to Helicobacter Pylori eradication and detection of API2-MALT1 fusion. *Am J Surg Pathol* 2004;28:1560-7.
48 Nakamura T, Seto M, Tajika M, *et al.* Clinical features and prognosis of gastric MALT lymphoma with special reference to responsiveness to H. pylori eradication and API2-MALT1 status. *Am J Gastroenterol* 2008;103:62-70.
49 Copie-Bergman C, Locher C, Levy M, *et al.* Metachronous gastric MALT lymphoma and early gastric cancer: is residual lymphoma a risk factor for the development of gastric carcinoma? *Ann Oncol* 2005;16:1232-6.
50 Boot H, de Jong D. Diagnosis, treatment decisions, and follow up in primary gastric lymphoma. *Gut* 2002;51:621-2.
51 El-Zimaity HM, Graham DY. Evaluation of gastric mucosal biopsy site and number for identification of Helicobacter pylori or intestinal metaplasia: role of the Sydney System. *Hum Pathol* 1999; 30:72-7.
52 Zauber NP, Berman EL. Synchronous and metachronous primary gastric lymphoma and adenocarcinoma: a clinicopathologic study of 12 patients. *Cancer* 1998;82:226-7.

# Chapter 4

## Pre-malignant gastric lesions in patients with gastric MALT lymphoma and metachronous gastric adenocarcinoma: a case-control study

Lisette G. Capelle[1], Caroline M. den Hoed[1], Annemarie C. de Vries[1], Katharina Biermann[2], Mariel K. Casparie[3], Gerrit A. Meijer[4], and Ernst. J. Kuipers[1,5]

[1]Departments of Gastroenterology and Hepatology, [2]Pathology and [5]Internal Medicine, Erasmus MC University Medical Center, Rotterdam, [3]Stichting PALGA, The Netherlands, [4]Department of Pathology, VU Medical Center, Amsterdam.

*Submitted*

## ABSTRACT

*Background:* Patients with gastric mucosa associated lymphoid tissue lymphoma (gMALT) or diffuse large B-cell lymphoma (DLBCL) have an increased risk of developing gastric carcinoma (GC). Identifying patients at high GC risk may lead to improved survival and prognosis. The aim of this case-control study was to evaluate whether atrophic gastritis (AG), intestinal metaplasia (IM) and dysplasia (DYS), can be identified in gMALT or DLBCL patients and whether these lesions are more severe in gMALT or DLBCL patients with a subsequent diagnosis of GC than in those without.

*Methods:* Patients with a first diagnosis of gMALT or DLBCL between 1991 and 2008 were identified in the Dutch nationwide histopathology registry (PALGA). Cases were patients with a diagnosis of gMALT or DLBCL and a subsequent diagnosis of GC. Controls were patients with a diagnosis of gMALT or DLBCL without a subsequent diagnosis of GC matched for age, sex and follow-up (fu). The baseline histopathology of these cases and controls was evaluated and scored for AG, IM and DYS according to the updated Sydney classification by an expert pathologist.

*Results:* In total 8 cases (M/F 3/5; mean fu 5.5 yrs; 1.1-7.4 yrs; gMALT/DLBCL 6/2) and 31 controls (M/F 16/15; mean fu 5.3 yrs; 0.7-11.9 yrs; gMALT/DLBCL 26/5) were included with a mean age of 60 years (range 18-86 years). At diagnosis, six (75%) cases had histological signs of premalignant gastric lesions; AG (12%), IM (38%), DYS (25%), whereas in the control group, 21 (68%) had histological evidence for premalignant gastric lesions; AG (23%), IM (35%), DYS (10%) at gMALT diagnosis (p=0.69). At GC diagnosis, 5 (63%) cases showed IM in the surrounding gastric mucosa. In 22 (71%) controls premalignant lesions were present at the end of follow up AG (19%), IM (45%), DYS (6%) (p=0.47).

*Conclusions:* No differences were demonstrated in the prevalence and severity of premalignant gastric lesions of cases and controls at gMALT or DLBCL diagnosis or at end of follow-up. In fact, premalignant gastric lesions were common in both cases and controls. This indicates that endoscopic and histopathologic surveillance with specific attention to the severity of premalignant gastric lesions after diagnosis and treatment of gastric lymphoma is warranted.

## INTRODUCTION

*Helicobacter pylori* is an important risk factor for gastric adenocarcinoma. It may even be a conditio sine qua non, since *H. pylori* was demonstrated in more than 90% of patients with gastric adenocarcinoma.[1,2] Due to this evident triggering of *H. pylori* in gastric carcinogenesis, the International Agency for Research on Cancer classified *H. pylori* as a class I carcinogen in 1994.[3]

*H. pylori* causes in the majority of patients a chronic inflammation of the gastric mucosa. This inflammation can progress via a widely accepted cascade initially proposed by Correa; from chronic inflammation to atrophic gastritis, to intestinal metaplasia, to dysplasia and eventually progress to gastric cancer.[4] Because of the low five-year survival rate of gastric cancer, early detection and surveillance of these lesions may improve gastric cancer prognosis.[5,6]

Besides gastric cancer, also marginal-zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) type (formerly known as low-grade MALT lymphoma (gMALT)) is strongly associated with *H. pylori* infection. For Diffuse Large B-Cell Lymphoma ((DLBCL), previously considered as high grade gastric MALT lymphoma) the association with a previous *H. pylori* infection remains controversial. In 1991, the first link between *H. pylori* and gastric MALT lymphoma was discovered and further studies demonstrated that gMALT regresses in 75% of the cases after *H. pylori* eradication as monotherapy.[7,8] Both gastric adenocarcinoma and gastric MALT lymphoma are thus long-term consequences of *H. pylori* infection, and we recently demonstrated in a Dutch nationwide study that patients with low and high grade gastric MALT lymphoma have a six times increased risk compared to the general Dutch population, of developing gastric cancer.[9]

In addition to these observations, previous studies reported that pre-malignant gastric lesions can also be observed in patients with gastric lymphoma.[9-12] Identification of these lesions in gMALT patients prior to a subsequent diagnosis of gastric cancer may improve surveillance and prognosis of these patients. However, whether pre-malignant gastric lesions can be used to identify patients with gastric lymphoma at highest risk for subsequent development of gastric cancer remains unknown. Therefore, the aim of this case-control study was to assess whether pre-malignant gastric lesions are more severe in gMALT or DLBCL patients with a subsequent diagnosis of gastric cancer compared to patients with gMALT or DLBCL lymphoma without a subsequent diagnosis of gastric cancer.

## METHODS

### *Histopathology database*

Histopathology and cytopathology reports were collected in a national archive (PALGA database) in the Netherlands. This archive has nationwide coverage since 1991.[13] Patients in this database are identified by date of birth, gender and the first 4 characters of their family name. This identification string enables the linkage of different tests belonging to the same patient, and therefore also to follow individual testing histories (dates and diagnoses) irrespective of the facility of treatment.[9,14]

All specimens receive a diagnostic code, similar to Systematized Nomenclature of Medicine (SNOMED) classification of the College of American Pathologists.[15] This code consists of a term indicating the anatomical location, type of sample, and a morphological term describing the finding. The records in the database contain these codes and the summary of the pathology report. In this study, data recorded in the PALGA database between 1991 and 2008 were included. For each report, gender, date of pathology report, summary text and diagnostic codes were made available.

### *Patient selection*

All patients with a histologically confirmed diagnosis of gMALT or DLBCL were identified in the PALGA database. Cases were patients with gMALT or DLBCL and a histologically confirmed diagnosis of gastric cancer. Controls were matched to cases by age and years of follow-up, and selected based on the fact that no diagnosis of gastric cancer was reported in their follow-up.

### *Histology*

An expert GI pathologist revised all biopsy specimens. The type and grade of the different stages of gastric pre-neoplastic changes in the tissue surrounding the gMALT or DLBCL or gastric carcinoma were classified according to the updated Sydney System classification.[16] If possible, the following items were evaluated separately: *H. pylori* density, acute inflammation (neutrophil infiltration), chronic inflammation (mononuclear infiltration), gastric glandular atrophy, and intestinal metaplasia. All these items were scored from 0 (absent), to 1 (mild), 2 (moderate), or 3 (marked). Dysplasia was assessed according to the revised Vienna classification.[16, 17]

### *Statistical analysis*

Cases were defined as patients with a gMALT or DLBCL and a subsequent diagnosis of gastric cancer. Controls were defined as patients with a gMALT or DLBCL with no subsequent diagnosis of gastric cancer. Continuous variables in cases and controls were compared using the Student's T-test. Categorical variables were compared using the Chi-squared test. A two sided p-value <0.05 was considered statistically significant.

## RESULTS

In total, 8 cases (3 males and 5 females) and 31 controls (16 males and 15 females) with a mean age of 60 years (range 18 to 86 years) were included. Gastric cancer occurred on average 5.5 years (range 1-7 years) after the initial diagnosis, whereas for controls the mean follow-up was 5.3 years (range 1-12 years). Six (75%) cases (M/F 3/3) demonstrated gMALT, and 2 (25%) cases (M/F 0/2) were diagnosed with DLBCL. In addition, 26 (84%) controls (M/F 13/13) demonstrated gMALT and 5 (16%) controls (M/F 3/2) a DLBCL. The baseline characteristics for the total cohort and for cases and controls with gMALT and DLBCL are presented in Table 1.

**Table 1.** Baseline characteristics of patients with gMALT and DLBCL

| | *Total* | | | *Low grade gastric MALT lymphoma* | | | *Diffuse Large B-Cell Lymphoma* | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Cases N=8 (%)** | **Controls N=31 (%)** | **p value** | **Cases N=6 (%)** | **Controls N=26 (%)** | **p value** | **Cases N=2 (%)** | **Controls N=5 (%)** | **p value** |
| **Gender** | | | | | | | | | |
| Male | 3 (37) | 16 (52) | 0.47 | 3 (50) | 13 (50) | 1.0 | 0 | 3 (60) | 0.15 |
| Female | 5 (63) | 15 (48) | | 3 (50) | 13 (50) | | 2 (100) | 2 (40) | |
| **Age** | | | | | | | | | |
| Mean (years) | 65.6 | 59.0 | 0.22 | 65.6 | 58.9 | 0.29 | 65.4 | 59.7 | 0.61 |
| Range (years) | 48.1-74.7 | 18.3-86.4 | | 48.1-74.7 | 18.3-86.4 | | 61.7-69.1 | 39.4-74.2 | |
| **Total follow-up** | | | | | | | | | |
| Mean (years) | 5.5 | 5.3 | 0.82 | 6.2 | 5.2 | 0.45 | 3.6 | 5.7 | 0.40 |
| Range (years) | 1.1-7.4 | 0.7-11.9 | | 4.1-7.4 | 0.7-11.9 | | 1.1-6.0 | 2.0-9.1 | |

### *Pre-malignant gastric lesions at gMALT or DLBCL diagnosis*

In three (37%) cases (gMALT or DLBCL) and 18 (58%) controls, chronic gastritis was identified in the surrounding tissue at diagnosis of gMALT or DLBCL (p=0.10). All cases with a chronic gastritis and 15 (48%) controls demonstrated an active component. The prevalence of pre-malignant gastric lesions is demonstrated in Figure 1. Overall, 75% of cases (gMALT and DLBCL) demonstrated pre-malignant lesions; atrophic gastritis (n=1), intestinal metaplasia (n=3) and low grade dysplasia (n=2) as most severe diagnosis. Within the controls, 68% dem-

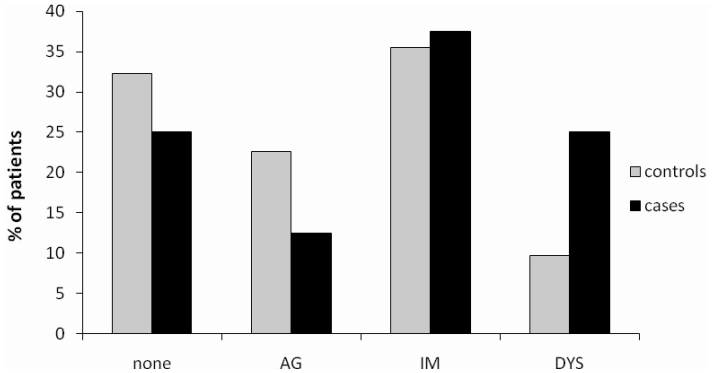


**Figure 1.** Prevalence of pre-malignant gastric lesion at gMALT or DLBCL diagnosis
Abbreviations: AG: atrophic gastritis; IM: intestinal metaplasia; DYS: dysplasia

onstrated these lesions; atrophic gastritis (n=7), intestinal metaplasia (n=11) and low grade dysplasia (n=3). One control with marked intestinal metaplasia also demonstrated a focus which was indefinite for dysplasia. The prevalence of pre-malignant gastric lesions was not significantly different between cases and controls (p=0.69).

### *Pre-malignant gastric lesions at gastric cancer diagnosis or end of follow-up*

At gastric cancer diagnosis, 6 (75%) cases with gMALT or DLBCL diagnosis and a subsequent diagnosis of gastric cancer were diagnosed with chronic gastritis in the surrounding tissue. In the remaining two cases, the tissue consisted solely of gastric cancer cells, therefore the surrounding tissue could not be evaluated. Twenty-six (84%) controls had histological signs of chronic gastritis (p=0.29). All cases and 8 (26%) controls with chronic gastritis showed an

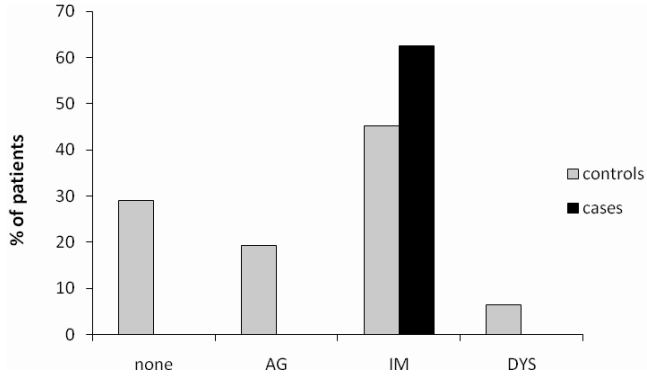


**Figure 2.** Prevalence of pre-malignant gastric lesions at gastric cancer diagnosis or end of follow-up
Abbreviations: AG: atrophic gastritis; IM: intestinal metaplasia; DYS: dysplasia

active component. At the end of follow-up, all cases demonstrated gastric cancer and 5 (63%) of them demonstrated intestinal metaplasia in the tissue surrounding the malignant cells. Within the control group, 6 (19%) controls demonstrated atrophic gastritis, 14 (45%) controls showed intestinal metaplasia and 2 (8%) controls had a diagnosis of low grade dysplasia (Figure 2). In 3 (10%) controls with moderate and marked intestinal metaplasia the biopsy specimens were classified as indefinite for dysplasia. No differences were demonstrated between the occurrence of pre-malignant gastric lesions identified in cases and controls at the end of follow-up (p=0.46).

### *Progression of pre-malignant gastric lesions*

On average gastric cancer was diagnosed within 5 years from initial diagnosis of gMALT or DLBCL. For controls, ten (32%) patients demonstrated progression of their pre-malignant lesions after a mean of 5.2 years. Three (33%) controls with no identified pre-malignant gastric lesion at diagnosis demonstrated after a mean follow-up of 6 years atrophic gastritis and five (16%) controls demonstrated intestinal metaplasia after a mean follow-up of 3 years. Two (6%) controls with a diagnosis of atrophic gastritis at diagnosis of their gMALT or DLBCL demonstrated after a mean of 7 years intestinal metaplasia as most severe pre-malignant lesion.

### *Gastric MALT or DLBCL*

Differences in prevalence of pre-malignant gastric lesions in patients with gMALT and DLBCL are presented in Table 2 and Table 3. The prevalence of pre-malignant gastric lesions at gMALT or DLBCL diagnosis was not significantly different between patients with gMALT and patients with DLBCL (p=0.93). In addition, no significant differences were demonstrated between the occurrence of pre-malignant gastric lesions between gMALT-cases and DLBCL- cases (p=0.45) or between gMALT-controls and DLBCL-controls (p=0.64) (Table 2). At the end of follow-up or gastric cancer diagnosis, no significant differences were demonstrated between patients with gMALT and those with DLBCL (Table 3).

**Table 2.** Prevalence of pre-malignant gastric lesions in gMALT and DLBCL at diagnosis

| | ***Total*** | | | *Cases* | | | *Controls* | | |
|---|---|---|---|---|---|---|---|---|---|
| | **gMALT n=32** | **DLBCL n=7** | **p - value** | gMALT n=6 | DLBCL n=2 | p - value | gMALT n=26 | DLBCL n=5 | p - value |
| None | **10** | **2** | **0.93** | 1 | 1 | 0.46 | 9 | 1 | 0.64 |
| AG | **6** | **2** | | 1 | 0 | | 5 | 2 | |
| IM | **12** | **2** | | 3 | 0 | | 9 | 2 | |
| DYS | **4** | **1** | | 1 | 1 | | 3 | 0 | |

Abbreviations: gMALT: gastric mucosa associated lymphoid tissue lymphoma; DLBCL: diffuse large B-cell lymphoma; AG: atrophic gastritis; IM: intestinal metaplasia; DYS: dysplasia

**Table 3.** Prevalence of pre-malignant gastric lesions in gMALT and DLBCL at gastric cancer diagnosis or end of follow-up

| | ***Total*** | | | *Cases* | | | *Controls* | | |
|---|---|---|---|---|---|---|---|---|---|
| | **gMALT n=32** | **DLBCL n=7** | **p - value** | gMALT n=6 | DLBCL n=2 | p - value | gMALT n=26 | DLBCL n=5 | p - value |
| None | **9** | **3** | **0.47** | 2 | 1 | 0.67 | 7 | 2 | 0.43 |
| AG | **4** | **2** | | 0 | 0 | | 4 | 2 | |
| IM | **17** | **2** | | 4* | 1* | | 13 | 1 | |
| DYS | **2** | **0** | | 0 | 0 | | 2 | 0 | |

*Intestinal metaplasia was identified in tissue surrounding the gastric cancer cells

Abbreviations: gMALT: gastric mucosa associated lymphoid tissue lymphoma; DLBCL: diffuse large B-cell lymphoma; AG: atrophic gastritis; IM: intestinal metaplasia; DYS: dysplasia

## DISCUSSION

This study shows that no differences exist between the prevalence and severity of pre-malignant gastric lesions in gastric lymphoma (gMALT and DLBCL) patients with a subsequent diagnosis of gastric cancer and those with no subsequent diagnosis of gastric cancer. Notably, the prevalence of moderate to severe pre-malignant gastric lesions such as intestinal metaplasia and dysplasia is substantial in both groups of patients, warranting careful surveillance of both gMALT and DLBCL patients not only for recurrence of lymphoma, but also for progression to adenocarcinoma.

Previous studies described varying prevalences of pre-malignant gastric lesions in patients with gastric lymphomas. For gMALT the prevalence of atrophic gastritis and intestinal metaplasia in the surrounding tissue ranged from 4-51% and 44-63% respectively.[12,18,19] The prevalence of dysplasia in gMALT patients remained less clear. Our observations for atrophic gastritis and intestinal metaplasia are in line with these previous studies. For dysplasia we demonstrate a considerable prevalence of 12% at gMALT diagnosis.

For diffuse large B-cell lymphomas, the prevalence of pre-malignant gastric lesions was unknown. In addition, the role of *H. pylori* in DLBCL remains controversial. *H. pylori* infection seems to play a role in the transformation from gMALT to DLBCL, however not all DLBCL seem to derive from gMALT. [20,21] Previous studies described a widely ranging prevalence of intestinal metaplasia in DLBCL, and very little data on the prevalence of dysplasia.[18,19] We observed a prevalence of 30% for intestinal metaplasia and 14% of dysplasia at DLBCL diagnosis. As our population of DLBCL patients was small, our findings are still associated with wide confidence intervals.

The high prevalence of pre-malignant gastric lesions in gMALT or DLBCL patients found in our study shows that both in gMALT patients as well as in DLBCL patients the histopathological pathway of pre-malignant gastric lesions is probably similar to the proposed cascade by Correa for intestinal type adenocarcinoma.[4] Moreover, these data suggest that *H. pylori* should be implicated in the etiology of both type of lymphoma, and strengthen the fact that gastric cancer risk is increased in lymphoma patients as published previously.[9,22,23]

This increased risk of gastric cancer adds to the need for intense endoscopic surveillance for the development of (pre)-malignant gastric lesions. A previous study described a rapid progression within 4 years of follow-up of pre-malignant gastric lesions in patients with gMALT. The authors showed a progression to atrophic gastritis in 91% and to intestinal metaplasia in 51%.[12] Our study showed a similar picture, but then already with a high prevalence of pre-malignant lesions at the time of lymphoma diagnosis. These findings suggest that the progression to gastric cancer in gastric MALT or DLBCL patients is accelerated compared to patients with no gastric lymphoma. This indicates that thoroughly scrutinizing the gastric mucosa at diagnosis as well as during surveillance for MALT or DLBCL is relevant as early detection of gastric cancer may reduce mortality. However, the yield of such an approach has to be confirmed by future prospective studies that include a larger population of patients with gMALT or DLBCL with long-term of follow-up.

Some limitations of this study warrant consideration. Firstly, the presence of *H. pylori* and extension of pre-malignant gastric lesions were difficult to evaluate. Previous studies have shown that the intragastric extent of intestinal metaplasia is an important risk factor for progression to gastric cancer and evaluating the severity and extent of this lesion by means of the OLGIM score results in adequate gastric cancer risk assessment.[24] Due to the retrospective design of this study, material from standardized biopsy sampling throughout the stomach was not routinely available. Thus, the exact extension of intestinal metaplasia and determination of individual OLGIM scores at baseline and follow-up was not feasible. In addition, *H. pylori* status and translocation status could not be evaluated in most biopsy specimens. These data are necessary for adequate prognosis assessment and accurate surveillance strategies, as previous studies demonstrated that the *API2-MALT1* t(11;18) chromosomal translocation results in increased dissemination to lymph nodes or other sites than the stomach.[25-27] Moreover, patients with this specific translocation are less responding to *H. pylori* eradication therapy.[27] Secondly, only a very small population of DLBCL patients was included, and even fewer patients developed gastric cancer. This implies that prevalences of premalignant lesions at baseline and incidences of gastric cancer during follow-up were associated with wide confidence intervals. However, paucity of literature on the prevalence of pre-malignant gastric lesions and the progression to gastric cancer in this patient category support the relevance of our data and our recommendations to consider that frequent surveillance of these patients should not only aim for the recurrence of lymphoma, but also for the progression to cancer.

In conclusion, although no differences were demonstrated in prevalence and severity of pre-malignant gastric lesions between gMALT or DLBCL patients with a subsequent diagnosis of gastric cancer and patients without a subsequent diagnosis of gastric cancer, the high prevalence of these lesions in both categories of patients and the association with subsequent cancer development warrant accurate endoscopic follow-up.

## REFERENCES


1 Uemura N, Okamoto S, Yamamoto S, *et al.* Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med* 2001;345:784-9.

2 Ekstrom AM, Held M, Hansson LE, *et al.* Helicobacter pylori in gastric cancer established by CagA immunoblot as a marker of past infection. *Gastroenterology* 2001;121:784-91.

3 Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. *IARC Monogr Eval Carcinog Risks Hum* 1994; 61:1-241.

4 Correa P. Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 1992;52:6735-40.

5 de Vries AC, Kuipers EJ. Epidemiology of premalignant gastric lesions: implications for the development of screening and surveillance strategies. *Helicobacter* 2007;12 Suppl 2:22-31.

6 de Vries AC, van Grieken NC, Looman CW, *et al.* Gastric cancer risk in patients with premalignant gastric lesions: a nationwide cohort study in the Netherlands. *Gastroenterology* 2008;134:945-52.

7 Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, *et al.* Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. *Lancet* 1991;338:1175-6.

8 Wotherspoon AC, Doglioni C, Diss TC, *et al.* Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of Helicobacter pylori. *Lancet* 1993;342:575-7.

9 Capelle LG, de Vries AC, Looman CW, *et al.* Gastric MALT lymphoma: epidemiology and high adenocarcinoma risk in a nation-wide study. *Eur J Cancer* 2008;44:2470-6.

10 Arista-Nasr J, Jimenez-Rosas F, Uribe-Uribe N, *et al.* Pathological disorders of the gastric mucosa surrounding carcinomas and primary lymphomas. *Am J Gastroenterol* 2001;96:1746-50.

11 Driessen A, Ectors N, Van Cutsem E, *et al.* Different gastritis features are linked to different gastric neoplasms. *Gastroenterol Clin Biol* 1999;23:747-53.

12 Lamarque D, Levy M, Chaumette MT, *et al.* Frequent and rapid progression of atrophy and intestinal metaplasia in gastric mucosa of patients with MALT lymphoma. *Am J Gastroenterol* 2006;101: 1886-93.

13 Casparie M, Tiebosch AT, Burger G, *et al.* Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. *Cell Oncol* 2007;29:19-24.

14 Van den Brandt PA, Schouten LJ, Goldbohm RA, *et al.* Development of a record linkage protocol for use in the Dutch Cancer Registry for Epidemiological Research. *Int J Epidemiol* 1990;19:553-8.

15 Cote RA, Robboy S. Progress in medical information management. Systematized nomenclature of medicine (SNOMED). *Jama* 1980;243:756-62.

16 Dixon MF, Genta RM, Yardley JH, *et al.* Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996;20:1161-81.

17 Dixon MF. Gastrointestinal epithelial neoplasia: Vienna revisited. *Gut* 2002;51:130-1.

18 Herrera-Goepfert R, Arista-Nasr J, Alba-Campomanes A. Pathologic features of the gastric mucosa adjacent to primary MALT-lymphomas. *J Clin Gastroenterol* 1999;29:266-9.

19 Driessen A, Ectors N, Creemers J, *et al.* Intestinal metaplasia in gastric malignancy: a comparison between carcinoma and lymphoma. *Eur J Gastroenterol Hepatol* 1998;10:595-600.

20 Wu XC, Andrews P, Chen VW, *et al.* Incidence of extranodal non-Hodgkin lymphomas among whites, blacks, and Asians/Pacific Islanders in the United States: anatomic site and histology differences. *Cancer Epidemiol* 2009;33:337-46.

21 Du MQ, Atherton JC. Molecular subtyping of gastric MALT lymphomas: implications for prognosis and management. *Gut* 2006;55:886-93.

22 Hamaloglu E, Topaloglu S, Ozdemir A, *et al.* Synchronous and metachronous occurrence of gastric adenocarcinoma and gastric lymphoma: A review of the literature. *World J Gastroenterol* 2006;12: 3564-74.

23 Morgner A, Miehlke S, Stolte M, *et al.* Development of early gastric cancer 4 and 5 years after complete remission of Helicobacter pylori associated gastric low grade marginal zone B cell lymphoma of MALT type. *World J Gastroenterol* 2001;7:248-53.

24 Capelle LG, de Vries AC, Haringsma J, *et al.* The staging of gastritis with the OLGA system using intestinal metaplasia as accurate alternative for atrophic gastritis. *Gastrointest Endosc* 2010;Accepted.

25 Nakamura T, Seto M, Tajika M, *et al.* Clinical features and prognosis of gastric MALT lymphoma with special reference to responsiveness to H. pylori eradication and API2-MALT1 status. *Am J Gastroenterol* 2008;103:62-70.

26 Ye H, Liu H, Attygalle A, *et al.* Variable frequencies of t(11;18)(q21;q21) in MALT lymphomas of different sites: significant association with CagA strains of H pylori in gastric MALT lymphoma. *Blood* 2003;102:1012-8.

27 Inagaki H, Nakamura T, Li C, *et al.* Gastric MALT lymphomas are divided into three groups based on responsiveness to Helicobacter Pylori eradication and detection of API2-MALT1 fusion. *Am J Surg Pathol* 2004;28:1560-7.

# Chapter 5

## Serum levels of leptin as marker for patients at high risk of gastric cancer

Lisette G. Capelle[1], Annemarie C. de Vries[1], Jelle Haringsma[1], Ewout W. Steyerberg[2], Caspar W. N. Looman[2], Nicole M. A. Nagtzaam[1], Herman van Dekken[3], Frank ter Borg[4], Richard A. de Vries[5], Ernst J. Kuipers[1,7]

[1]Departments of Gastroenterology and Hepatology, [2]Public Health, [3]Pathology and [7]Internal Medicine, Erasmus MC University Medical Center, Rotterdam, [4]Department of Hepato-gastroenterology, Deventer Hospital, Deventer, [5]Department of Internal Medicine, University Medical Center Groningen, Groningen.

*Helicobacter; 2009;14(6):596-604*

## ABSTRACT

*Background:* Serological screening for gastric cancer (GC) may reduce mortality. However, optimal serum markers for advanced gastric precursor lesions are lacking.

*Aim:* To evaluate in a case–control study whether serum leptin levels correlate with intestinal metaplasia (IM) and can serve as a tool to identify patients at high risk for GC.

*Materials and Methods:* Cases were patients with a previous diagnosis of IM or dysplasia, controls were patients without such a diagnosis. All patients underwent endoscopy. Fasting serum was collected for the measurement of leptin, pepsinogens I/II, gastrin, and *Helicobacter pylori*. Receiver operating characteristic (ROC) curves and their area under the curve (AUC) were provided to compare serum leptin levels with other serological markers.

*Results:* One hundred nineteen cases and 98 controls were included. In cases, the median leptin levels were 116.6 pg/mL versus 81.9 pg/mL in controls ($p = .01$). After adjustment for age, sex and BMI, leptin levels remained higher in cases than in controls ($p < .005$). In multivariate analysis, male sex ($p = .002$), age (<0.001), low pepsinogen levels ($p = .004$) and high leptin levels ($p = .04$) were independent markers for the presence of IM. In addition, a ROC curve including age, sex and pepsinogen I levels had an AUC of 0.79 (95% CI (0.73–0.85)). Adding serum leptin levels increased the AUC to 0.81 (95% CI (0.75–0.86)).

*Conclusions:* High leptin levels are associated with an increased risk of IM. Moreover, serum leptin levels are a significant independent marker for the presence of IM. However, in combination with the serological test for pepsinogen I the additional value of serum leptin levels is rather limited.

## INTRODUCTION

Gastric cancer (GC) is the fourth most common cancer and second leading cause of cancer-related death worldwide.[1] As symptoms are often absent, GC is usually diagnosed at an advanced stage with limited curative options.[2] To reduce its mortality rate, population-based screening programs have been implemented in high incidence countries, such as Japan.[3-5] During the last decades, serological screening has been introduced as part of these programs, as serological markers were demonstrated to predict GC development.[6-8]

In particular for the diagnosis of atrophic gastritis, serological testing for a combination of pepsinogens I and II, gastrin and *Helicobacter pylori* antibodies have yielded accurate results with in particular a high specificity (95–98%).[9-11] However, the value of these tests for the diagnosis of more advanced precursor lesions, i.e. intestinal metaplasia (IM) and dysplasia, was much lower with a sensitivity of 66% and 70% and specificity of 78% and 65%, respectively.[12] Therefore, better markers are needed for a prediction of more advanced precursor lesions.

The common denominator of several serological markers is their release by specialized cells of the stomach lining. Based on this characteristic, leptin has been identified as potential new serological marker for pathological conditions of the stomach. Leptin is a 16-kDa peptide hormone that plays a key role in appetite regulation as well as energy homeostasis.[13] It is primarily produced by adipocytes, but it has been shown that, similar to pepsinogen I, gastric chief cells are also a source of leptin.[14,15] Both sources of leptin can have an effect on serum leptin levels. However, it remains unknown whether serum leptin levels can fulfill the role of a new serologic marker in the gastric carcinogenesis. As the increased expression of leptin has been associated with several types of cancer and leptin is produced by the gastric mucosa, we hypothesized that serum leptin levels may have a similar role as pepsinogen I as serum marker for the identification of subjects at risk of GC.[16-17] Serum levels of pepsinogen I increase with gastric inflammation and decrease with progressive severity of atrophy.[18] In addition, several immuno-histochemical staining studies showed that leptin levels increased due to *H. pylori* infection.[19,20] However, neither studies investigated the serum leptin levels in patients with more advanced precursor lesions, nor whether a combination of serum leptin and pepsinogen levels improves the efficacy of non-invasive screening.

Therefore, the aim of this study was to evaluate whether there is an association between serum levels of leptin and the presence of gastric IM and dysplasia, and whether serum leptin levels can serve as a tool to identify patients at high risk of GC. Therefore, we compared the serum levels of leptin in patients with IM and dysplasia with those of patients without these pre-malignant gastric lesions.

## MATERIAL AND METHODS

### *Case selection*

Consecutive patients with a diagnosis of IM and/or dysplasia of the gastric mucosa based on histology according to the updated Sydney biopsy protocol, were eligible for inclusion. All these patients were invited to participate in this study. Biopsy specimens of the baseline endoscopy were revised by an expert pathologist specialized in GI pathology. Patients with a confirmed diagnosis of IM and/or dysplasia after revision were included after informed consent. All these patients underwent a surveillance endoscopy between March 2007 and March 2008. Patients with a diagnosis or history of esophageal varices, upper gastrointestinal malignancy, or esophageal or gastric surgery were excluded.

### *Control Population*

We recruited consecutive outpatients ≥18 years of age undergoing routine upper endoscopy for any indication at the endoscopy unit at the Erasmus MC. Patients with a previous diagnosis of IM and dysplasia were excluded, as well as patients with a diagnosis or history of esophageal varices, upper gastrointestinal malignancy, esophageal, or gastric surgery. Patients were also excluded in case gastric biopsy specimens at the study endoscopy demonstrated IM or dysplasia.

### *Endoscopy*

Demographic and clinical information were collected from cases and controls by means of a structured questionnaire prior to endoscopy. All patients completed upper gastrointestinal endoscopy using a standard forward-viewing video gastroscope (Olympus GIF Q160; Olympus optical Co., Tokyo, Japan). In cases, extensive biopsies for histological assessment were taken from five standardized intragastric locations: four biopsies from the antrum (2–3 cm proximal to the pylorus; one of each quadrant), two from the angulus, four from the midcorpus (two from the lesser curvature, two from the greater curvature), and two from the cardia (just below the gastro-oesophageal junction). In case of endoscopically visible lesions, additional targeted biopsy samples were obtained.

In controls, five random biopsy samples were obtained for histology according to the Sydney classification [21]; two from the antrum, two from the corpus (one from the lesser curvature, one from the greater curvature), and one from the angulus.

### *Histological Assessment*

Biopsy specimens obtained from the stomach were fixed in buffered formalin and embedded in paraffin. The specimens were stained by hematoxylin and eosin. An expert GI pathologist blinded to endoscopic and clinical findings reviewed all sections. The type and grade of the different stages of the gastric carcinogenesis was classified according to the updated Sydney System classification. The following items were evaluated separately: *H. pylori* density, acute inflammation (neutrophil infiltration), chronic inflammation (mononuclear infiltration), gastric glandular atrophy, and IM. All these items were scored from 0 (absent), to 1 (mild), 2 (moderate), or 3 (marked).[21] Dysplasia was assessed according to the revised Vienna classification.[21,22] Extensive IM was similar to previous studies defined as either IM in the random biopsies from at least two different intragastric locations (multifocal IM) or moderate or marked IM in at least two random biopsies (severe grades of IM).[23] These definitions of IM have turned out to be useful parameters to predict high GC risk.[23]

### *Serologic Markers*

Fasting serum was collected from all patients. The samples were collected and stored in aliquots at −80 °C until analysis. Serum leptin was measured using a commercial enzyme immunoassay from R & D systems, this immunoassay has been shown to quantitate leptin highly accurate and has excellent results in terms of reproducibility and repeatability.[24] The serological markers pepsinogen I and II, gastrin, *H. pylori* antibodies, and CagA status were measured using commercial enzyme immunoassays from Biohit (Helsinki, Finland), Orion Diagnostica (Espo, Finland), and Ravo Diagnostica (Freiburg, Germany), respectively. All tests were performed according to the instructions of the manufacturers.

### *Statistical Analysis*

Subjects with a previously confirmed diagnosis of IM or dysplasia were considered as cases. Subjects with no histologically identified IM or dysplasia at gastroscopy and with no previous diagnosis of IM or dysplasia were considered to be controls. In the absence of data on serum leptin levels in cases with IM versus controls, we were unable to make an a priori power calculation. Therefore, a considerable number of cases and matched controls were included for accurate and safe conclusions on clinically relevant differences between both groups.

Continuous variables in cases and controls were compared using the Student's t-test. Categorical variables were compared using the chi-squared test. A two-sided p-value <.05 was considered statistically significant. Considering the substantial loss of power, continuous variables were, if possible, not dichotomized.[25] All continuous variables were tested for linearity. Pepsinogen I, the rate of pepsinogen I/II, gastrin and leptin were log-transformed

to approach normal distribution. Linear regression was used to describe and test differences in leptin levels between cases and controls. The effect of possible confounders on the differences was tested by adding these variables to the model (age, sex, and BMI). We imputed BMI in 15% of cases on the assumption of MAR (missing at random) dependent on age and sex.[26] To estimate the predictive power of clinical and serological markers for the presence of IM, univariate, and multivariate logistic regression analyses were performed. For the presence of extensive IM, we used multivariate polytomous logistic regression analysis. Odds ratios with 95% confidence intervals (CIs) were used as a measure of association.

In the logistic regression analysis, the effects of log-transformed variables (serum levels of pepsinogens, gastrin, and leptin) were evaluated for a twofold increase of these variables on the presence of IM. In addition, receiver operating characteristic (ROC) curves and their area under the curve (AUC) were provided to compare serum leptin levels with other serological markers, and to determine best cut-off values. At every cut-off in the ROC curve the sum of the sensitivity and specificity was calculated (Youden's index), the highest sum was used as best cut-off value for serum leptin levels.[27]

## RESULTS

### *Study Population*

In total, 119 cases and 98 controls (108 males, 109 females) with a mean age of 55 years (range 18–81) were included. The majority of cases (73.3%) were of Dutch descent. The baseline characteristics of the cases and controls are presented in Table 1. Five controls accepted to participate, but refused to fulfill the questionnaire. As a result, demographic data of these patients are missing. Patients with IM were significantly older (mean age 60 years, range 23–81) than control subjects (mean age 48 years, range 18–76) ($p < .001$). Sex, BMI, and the prevalence of smoking, alcohol use and proton pump inhibitor (PPI) use did not significantly differ between cases and controls (Table 1). Overall, 41 cases and 38 controls had histological signs of *H. pylori* colonization ($p = .46$). None of the *H. pylori*-negative controls, but 32 of the *H. pylori*-negative cases had previously successfully been treated with *H. pylori* eradication therapy. The mean duration between *H. pylori* eradication and surveillance endoscopy was 5 years (range 0–20).

### *Serum Leptin Levels*

The mean serum leptin levels were 116.6 pg/mL (interquartile range (IQR) 75.0–207.5) in cases and 81.9 pg/mL (IQR 32.9–207.0) in controls ($p = .01$). The relation between serum leptin levels and possible influencing variables is presented in Table 2. Serum leptin levels

**Table 1.** Baseline characteristics

| | Cases N= 119 (%) | Controls N= 98 (%) | p value for difference |
|---|---|---|---|
| **Mean age** | 60.4 | 48.3 | <0.001 |
| **Sex** | | | 0.07 |
| - Male | 66 (55) | 42 (43) | |
| - Female | 53 (45) | 56 (57) | |
| **Mean BMI** | 26.3 (77) | 25.7 (95) | 0.37 |
| **Ethnicity** | | | 0.02 |
| - Dutch | 95 (80) | 64 (65) | |
| - Middle East | 10 (8) | 14 (14) | |
| - Remaining | 14 (12) | 15 (15) | |
| - Missing | | 5 (5) | |
| **Smoking** | | | 0.63 |
| - Non-smoker | 53 (45) | 49 (50) | |
| - Smoker | 22 (18) | 14 (14) | |
| - Missing | 44 (37) | 35 (36) | |
| **Alcohol use** | | | 0.46 |
| - <1unit/day | 21 (18) | 17 (17) | |
| - ≥1 unit/day | 39 (33) | 25 (26) | |
| - Missing | 59 (49) | 56 (57) | |
| **Proton Pump Inhibitor (PPI)** | | | 0.14 |
| - No PPI | 51 (43) | 44 (45) | |
| - PPI | 67 (56) | 49 (50) | |
| - Missing | 1 (1) | 5 (5) | |
| ***H. pylori*** | | | 0.46 |
| - Negative | 78 (66) | 60 (61) | |
| - Positive | 41 (34) | 38 (39) | |
| **CagA** | | | 0.89 |
| - Negative | 74 (62) | 60 (61) | |
| - Positive | 45 (38) | 38 (39) | |
| **Mean of pepsinogen I levels*** | 92.1 | 143.8 | 0.001 |
| **Mean of pepsinogen I/II*** | 6.9 | 10.8 | <0.001 |
| **Mean of gastrin levels*** | 10.5 | 6.9 | 0.06 |
| **Mean of serum leptin levels*** | 116.6 | 81.9 | 0.01 |

* Means of serum markers were calculated based on log-transformed data

increased with age and higher BMI (both $p < .001$). Moreover, serum leptin concentration was 2.9 times higher in female subjects than in male subjects ($p < .001$). Current smokers had a serum leptin concentration 0.7 times lower than subjects who were currently not smoking

**Table 2.** Relation between serum leptin levels and possibly influencing variables in 217 subjects (cases and controls)

| | Total N= 217 (%) | Mean of serum leptin levels* | Odds ratio [95% CI]** | p value |
|---|---|---|---|---|
| **Age (range 18-81 yrs)** | | 99.4 | 1.02 [1.001-1.03]*** | 0.001 |
| **Sex** | | | | <0.001 |
| - Male | 109 (50) | 58.4 | 1.0 | |
| - Female | 108 (50) | 168.5 | 2.88 [2.25-3.70] | |
| **BMI** | 187 (86)[#] | 101.5 | 1.14 [1.11-1.17][§] | <0.001 |
| **Ethnicity** | | | | 0.48 |
| - Dutch | 159 (73) | 93.3 | 1.0 | |
| - Middle East | 24 (11) | 107.0 | 1.15 [0.72-1.81] | |
| - Remaining | 29 (13) | 126.4 | 1.35 [0.89-2.07] | |
| - Missing | 5 (2) | 130.1 | 1.39 [0.54-3.61] | |
| **Smoking** | | | | 0.05 |
| - Non-smoker | 102 (47) | 119.4 | 1.0 | |
| - Smoker | 36 (17) | 79.9 | 0.67 [0.45-1.00] | |
| - Missing | 79 (36) | 86.8 | 0.73 [0.53-0.99] | |
| **Alcohol use** | | | | 0.44 |
| - <1 unit/day | 38 (18) | 105.4 | 1.0 | |
| - ≥1 unit/day | 64 (29) | 86.2 | 0.82 [0.53-1.26] | |
| - Missing | 115 (53) | 105.6 | 1.00 [0.68-1.48] | |
| **PPI use** | | | | 0.14 |
| - No PPI | 95 (44) | 86.0 | 1.0 | |
| - PPI | 116 (53) | 109.2 | 1.27 [0.95-1.69] | |
| - Missing | 6 (3) | 162.1 | 1.88 [0.78-4.53] | |
| ***H. pylori*** | | | | 0.25 |
| - Negative | 138 (64) | 105.7 | 1.0 | |
| - Positive | 79 (36) | 89.8 | 0.84 [0.62-1.13] | |
| **CagA** | | | | 0.76 |
| - Negative | 134 (62) | 97.7 | 1.0 | |
| - Positive | 83 (38) | 102.3 | 1.05 [0.78-1.40] | |
| **Serum pepsinogen I levels (two fold)[§§]** | | 99.4 | 0.96 [0.87-1.05][§§] | 0.38 |
| **Serum pepsinogen I/II (two fold)[§§]** | | 99.4 | 0.94 [0.84-1.05][§§] | 0.30 |
| **Serum gastrin levels (two fold)[§§]** | | 99.4 | 1.13 [1.07-1.20][§§] | <0.001 |

* Means of serum leptin levels were calculated based on log-transformed data; ** Odds ratio were calculated by univariate linear regression analysis; *** The odds ratio increased by 1.09 in case age increased by a value of one year; [#] In 14% of cases BMI was imputed on the assumption of MAR (missing at random) dependent on age and sex; [§]The odds ratio increased by 1.14 in case BMI increased by a value of one, [§§]In case serum markers increased twofold the odds ratio increased (gastrin) or decreased (pepsinogen I and pepsinogen I/II rate)

(p = .05). No significant correlations were demonstrated between serum leptin levels and ethnicity, alcohol use, and PPI use (Table 2). In addition, neither were serum leptin levels significantly associated with *H. pylori* infection, nor were significant alterations in serum leptin levels observed between eradicated cases and noneradicated cases (serum leptin levels 129.2 and 102.4 pg/mL, respectively) (p = .25).

### *Serum Leptin Levels and Intestinal Metaplasia*

In the case population, all cases had a previous histologically confirmed diagnosis of IM and/or dysplasia. However, in 32 (27%) of the cases the presence of IM was not confirmed during the study surveillance endoscopy. In the remaining 87 (73%) cases, the presence of IM was confirmed histologically during study surveillance endoscopy. Within this group, the most severe grade of IM was mild in 16 (18%) cases, moderate in 24 (28%) cases, and marked in 47 (54%) cases. Gastric dysplasia was detected in 5 (4%) cases.

Serum leptin levels were not significantly different between cases with mild-IM (mean leptin levels 156.0 pg/mL), moderate IM (mean leptin levels 146.4 pg/mL) or severe IM (mean leptin levels 98.9 pg/mL). In addition, no significant differences were demonstrated in serum leptin levels between cases with IM (mild, moderate, or marked) or cases with dysplasia (mean leptin levels 96.4 pg/mL) (p = .64).



**Table 3.** Multivariable logistic regression analysis: Relation between intestinal metaplasia (IM) and serological markers, adjusted for age and gender (n=217)

| | Univariate analysis Odds ratio [95% CI] IM (n=119) vs controls | p | Multivariate analysis Odds ratio [95% CI] IM (n=119) vs controls | p |
|---|---|---|---|---|
| **Age (year)** | 1.1 [1.04-1.1] | <0.001 | 1.1 [1.04-1.1] | <0.001 |
| **Gender (male)** | 1.7 [0.9-3.0] | 0.09 | 3.4 [1.5-7.4] | 0.002 |
| ***H. pylori* (positive)** | 0.6 [0.3-1.2] | 0.16 | 0.5 [0.3-1.1] | 0.11 |
| **CagA (positive)** | 0.8 [0.5-1.6] | 0.60 | 1.0 [0.5-2.1] | 0.90 |
| **Serum pepsinogen I levels* (two fold)#** | 0.3 [0.1-0.5] | <0.001 | 0.3 [0.1-0.7] | 0.004 |
| **Serum pepsinogen I/II* (two fold)#** | 0.4 [0.2-0.7] | 0.004 | 0.6 [0.2-1.5] | 0.33 |
| **Serum gastrin levels* (two fold)#** | 1.2 [0.9-1.7] | 0.24 | 1.0 [0.7-1.5] | 0.81 |
| **Serum leptin levels* (two fold)#** | 2.0 [1.1-3.5] | 0.01 | 1.9 [1.0-3.6] | 0.04 |

*serum leptin, pepsinogen I, pepsinogen I/II and gastrin were calculated based on log-transformed data; #In case serum levels would be two fold increased the risk of IM changes by the Odds rati

In an age- and sex-adjusted analysis, we found that patients with IM have a significantly increased serum leptin concentration compared to controls (OR 2.0 (95% CI 1.1–3.5)) (Table 3). After further adjusting for BMI, the odds ratio was 2.9 (95% CI 1.4–6.0) in patients with a twofold increase in leptin levels (p = .005). In male subjects, the association between IM and high leptin levels was strong (OR 5.1 (95% CI 2.3–14.4) (p = .002)), but no significant association between high serum leptin levels and IM was found in women (OR 1.2 (95% CI 0.4–4.1) (p = .71)). However, testing this difference by way of an interaction term demonstrated no significant difference between males and females (p = .13).

Within multivariate analysis, male sex, age, low pepsinogen I levels, and a high leptin concentration were identified as independent risk factors for a diagnosis of IM (Table 3). Gastrin was no independent predictor for IM. The pepsinogen I/II ratio showed a significant association to IM in univariate analysis, but was of no additional value in multivariate analysis. Low serum pepsinogen I levels were the best serologic marker for the prediction of the presence of IM (OR 0.3, 95% CI 0.1–0.7, p = .004), but leptin levels were of significant additional value (OR 1.9, 95% CI 1.0–3.6, p = .04). In addition, a ROC curve including the variables age, gender, and pepsinogen I levels had an AUC of 0.79 (95% CI (0.73–0.85)). Adding serum leptin levels to this model increased the AUC to 0.81 (95% CI (0.75–0.86)).

Best cut-off values for both serum pepsinogen I levels and serum leptin levels were evaluated by Youdens index [27]. A best cut-off value of 50 µg/mL was calculated for pepsinogen I levels and a serum leptin level of 94 pg/mL was determined for leptin as best cut-off value. These best cut-off values evaluated by Youdens index resulted in an AUC value of 0.81 (95% CI (0.75–0.86)) for age, gender, and a pepsinogen I levels (50 µg/mL) and an increase of 0.02 to an AUC of 0.83 (95% CI (0.77–0.88)) for age, gender, pepsinogen (50 µg/mL) and leptin levels (94 pg/mL).

### *Extensive Intestinal Metaplasia*

Within the case population, 68 (57%) patients demonstrated IM in the random biopsies from at least two different intragastric locations, which was defined as extensive IM (multifocal IM), and 54 (45%) patients had moderate or marked IM in two or more biopsies (severe grades of IM). Table 4 demonstrates that age, gender, and high serum leptin levels are independent predictors for patients with focal (nonextensive) IM (AUC 77%), whereas for patients with multifocal (extensive) IM, leptin levels are no independent predictor (AUC 85%). In patients with severe grades of IM, serum leptin levels were no independent predictor (data not shown).

**Table 4.** Multivariable polytomous logistic regression analysis: Relation between extensive or non-extensive intestinal metaplasia (IM) and serological markers

| | Non-extensive IM (n=51) vs. controls Odds ratio [95% CI] | p | Extensive IM (n=68) vs. controls Odds ratio [95% CI] | p |
|---|---|---|---|---|
| **Age (year)** | 1.05 [1.02-1.08] | 0.003 | 1.1 [1.06-1.1] | <0.001 |
| **Gender (male)** | 5.2 [1.9-14.3] | 0.002 | 2.2 [0.8-6.1] | 0.14 |
| ***H. pylori* (positive)** | 0.6 [0.3-1.6] | 0.33 | 0.7 [0.3-1.7] | 0.42 |
| **CagA (positive)** | 1.0 [0.4-2.3] | 0.94 | 1.0 [0.4-2.4] | 0.98 |
| **Serum pepsinogen I levels* (two fold)#** | 0.7 [0.2-2.0] | 0.48 | 0.2 [0.1-0.5] | 0.001 |
| **Serum pepsinogen I/II* (two fold)#** | 0.9 [0.3-2.7] | 0.85 | 0.6 [0.2-2.0] | 0.46 |
| **Serum gastrin levels* (two fold)#** | 0.8 [0.5-1.3] | 0.33 | 1.5 [0.9-2.6] | 0.13 |
| **Serum leptin levels* (two fold)#** | 3.2 [1.5-7.2] | 0.003 | 1.5 [0.7-3.4] | 0.28 |

*serum leptin, pepsinogen I, pepsinogen I/II and gastrin were calculated based on log-transformed data; #In case serum levels would be two fold increased the risk of IM changes by the Odds ratio



## DISCUSSION

This case–control study shows that serum leptin levels are significantly higher in patients with IM of the stomach than in controls. In combination with the established risk factors, in particular male sex, advancing age, and low serum pepsinogen I levels, serum leptin levels can serve as an extra tool to predict IM (Table 3). However, our results show that the additional value of this noninvasive marker is rather low.

As histology has important limitations, such as sampling effects and interobserver variability, noninvasive screening methods for GC have been tested for decades.[28] These methods usually included pepsinogen I and pepsinogen II, their ratio, gastrin, and *H. pylori* serology, which showed moderate to high specificities and a high predictive value for atrophic gastritis.[9,11,29-31] In Japan, these serological tests have therefore been introduced in the nationwide screening programs. However, there is still a need for markers for more advanced precursor lesions that improve the efficacy of noninvasive screening. Gastric leptin is similar to pepsinogen I produced by the chief cells in the gastric mucosa and may therefore serve as a new serologic marker for the identification of patients with high GC risk.

In this study, we show that serum leptin levels correlate with the presence or absence of IM and have an additional value in detecting IM. Similar to pepsinogen I, serum leptin levels increase with inflammation of the gastric mucosa, and seem to decrease in patients with more severe IM and dysplasia, presumably because of the associated progressive atrophy of the stomach lining. However, compared to pepsinogen I, serum leptin levels show much more variability within individuals and between males and females resulting in insufficient performance as serological marker. Although we demonstrate that leptin is a better marker for IM than gastrin, low serum pepsinogen I levels remain the best independent serological predictor for IM (Table 3). Combining the serological tests for pepsinogen and leptin has limited additional value compared to pepsinogen testing alone, while at the same time increasing screening costs. Therefore, although serum leptin levels are of significant additional value in predicting gastric IM, measuring leptin levels seems not very useful in clinical practice for screening purposes in patients at risk for GC.

Previous studies demonstrated that serum leptin levels were significantly lower in males than in females.[32,33] This sex-related difference could be explained by the fact that estrogens stimulate leptin production, whereas testosterone inhibits the production.[16] Also, leptin correlates with fat cell volume, the cell size and distribution of adipocytes. Subcutaneous adipocytes are larger than omental adipocytes and produce more leptin. The larger subcutaneous adipose tissue mass relative to omental mass in females compared to males thus results in more leptin production.[34] These observations are in line with our study, in which males had significant lower leptin levels than females. In addition, although not significantly different, males tended to have a stronger association between the risk of IM and high serum leptin levels than females. As a consequence of this, serum leptin levels might be a marker of additional value particularly in males, as was also described in a previous study.[32] However, these findings should be further evaluated in a larger population of male patients with premalignant gastric lesions.

As *H. pylori* eradication can result in changes in body weight, leptin levels are probably affected by *H. pylori* eradication. However, the correlation between *H. pylori* infection and leptin levels remains controversial. Although immuno-histochemical studies demonstrated that gastric mucosal leptin expression and secretion were significantly increased in *H. pylori*-positive patients compared to *H. pylori*-negative patients, serum leptin levels did not significantly differ between these patients. In addition, *H. pylori* eradication had no significant effect on serum leptin levels.[19,20,35-37] In line with these previous observations, the 32 eradicated cases in our study did not show significantly altered serum leptin levels compared to the noneradicated cases.

In this study, we used IM as a marker of GC risk. This approach is based on various older and more recent cohort studies comparing the GC incidence in subjects with and without IM at baseline. We recently described in a nationwide study that approximately 2% of patients with IM developed invasive GC within 10 years of follow-up.[38] This risk further increased con-

siderably in those subjects who had additional signs of dysplasia.[38] For the development of optimal screening and surveillance strategies, patients at high risk of progression to GC need to be identified. Previous studies have shown that not only the most severe grading, but also the intragastric extent of IM is an important marker for progression to GC and that pepsinogen levels can assess the extent of atrophic gastritis as well as IM.[9,12,23,39,40] This observation is consistent with our study in which the pepsinogen I ratio was significantly decreased in patients with extensive IM (Table 4). However, serum leptin levels were of no additional value in diagnosing extensive IM (Table 4).

This study described the association between serum leptin levels and IM. However, our study design does not permit evaluating the exact mechanisms for the differences in leptin levels we found. Potential weaknesses of our study are firstly that gastric IM is multifocal and often indistinguishable by endoscopy. These factors make the results subject to sampling error. To overcome this limitation, we obtained multiple gastric biopsies from five different intragastric locations.

Despite this approach, the presence of IM was not confirmed in 27% of the case population during the study surveillance endoscopy. Similar observations have been made in previous studies.[23,41] Excluding this group of patients from the analysis resulted in higher leptin levels in cases (mean serum leptin levels of 119.7 pg/mL), with a similar association between serum leptin levels and the presence of IM ($p = .01$). On the other hand, a previous study has shown that biopsy sampling according to the updated Sydney classification is likely to underestimate the true prevalence of IM in some 10% of cases (A. C. de Vries, unpublished data). This implies that some of our controls were likely to have had undiagnosed IM. If so, this would further strengthen the true correlation between increased serum leptin levels and IM.

Second, the majority of cases and controls were overweight. Despite controlling for possible confounders, unanticipated variables, including medical conditions, comorbidities or medication use could have influenced the serum leptin levels in these patients. In addition, although serum leptin levels were adjusted to BMI, diet effects could have influenced serum leptin levels. Future studies using structured questionnaires are necessary to evaluate the role of these possible confounders and the effect on serum leptin levels.

The strength of the study lies in the inclusion of a large cohort of patients with IM, the confirmation of these preneoplastic conditions by an expert pathologist, and the inclusion of a group of controls with no previous or current diagnosis of a premalignant gastric lesion or GC.

In conclusion, serum leptin levels are significantly higher in patients with IM and can serve as an independent predictor for IM. However, in combination with age, gender, and pepsinogen I levels the additional value of leptin measurements for the presence of IM is rather limited.

## REFERENCES


1 Fox JG, Wang TC. Inflammation, atrophy, and gastric cancer. *J Clin Invest* 2007;117:60–69
2 Bowles MJ, Benjamin IS. ABC of the upper gastrointestinal tract: cancer of the stomach and pancreas. *BMJ* 2001;323:1413–6
3 Hosokawa O, Miyanaga T, Kaizaki Y, et al. Decreased death from gastric cancer by endoscopic screening: association with a population-based cancer registry. *Scand J Gastroenterol* 2008;43: 1112–5
4 Lee KJ, Inoue M, Otani T, et al. Gastric cancer screening and subsequent risk of gastric cancer: a large-scale population-based cohort study, with a 13-year follow-up in Japan. *Int J Cancer* 2006; 118:2315–21
5 Oshima A, Hirata N, Ubukata T, et al. Evaluation of a mass screening program for stomach cancer with a case-control study design. *Int J Cancer* 1986;38:829–33
6 Yoshihara M, Hiyama T, Yoshida S, et al. Reduction in gastric cancer mortality by screening based on serum pepsinogen concentration: a case-control study. *Scand J Gastroenterol* 2007;42:760–4
7 Leung WK, Wu MS, Kakugawa Y, et al. Screening for gastric cancer in Asia: current evidence and practice. *Lancet Oncol* 2008;9:279–87
8 Miki K, Morita M, Sasajima M, et al. Usefulness of gastric cancer screening using the serum pepsinogen test method. *Am J Gastroenterol* 2003;98:735–9
9 Vaananen H, Vauhkonen M, Helske T, et al. Non-endoscopic diagnosis of atrophic gastritis with a blood test. Correlation between gastric histology and serum levels of gastrin-17 and pepsinogen I: a multicentre study. *Eur J Gastroenterol Hepatol* 2003;15:885–9
10 Storskrubb T, Aro P, Ronkainen J, et al. Serum biomarkers provide an accurate method for diagnosis of atrophic gastritis in a general population: The Kalixanda study. *Scand J Gastroenterol* 2008; 43:1–8
11 Watabe H, Mitsushima T, Yamaji Y, et al. Predicting the development of gastric cancer from combining Helicobacter pylori antibodies and serum pepsinogen status: a prospective endoscopic cohort study. *Gut* 2005;54:764–8
12 Dinis-Ribeiro M, da Costa-Pereira A, Lopes C, et al. Validity of serum pepsinogen I/II ratio for the diagnosis of gastric epithelial dysplasia and intestinal metaplasia during the follow-up of patients at risk for intestinal-type gastric adenocarcinoma. *Neoplasia* 2004;6:449–56
13 Zhang Y, Proenca R, Maffei M, et al. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994;372:425–32
14 Francois F, Roper J, Goodman AJ, et al. The association of gastric leptin with oesophageal inflammation and metaplasia. *Gut* 2008;57:16–24
15 Bado A, Levasseur S, Attoub S, et al. The stomach is a source of leptin. *Nature* 1998;394:790–3
16 Garofalo C, Surmacz E. Leptin and cancer. *J Cell Physiol* 2006;207:12–22
17 Ishikawa M, Kitayama J, Nagawa H. Expression pattern of leptin and leptin receptor (OB-R) in human gastric cancer. *World J Gastroenterol* 2006;12:5517–22
18 de Vries AC, Haringsma J, Kuipers EJ. The detection, surveillance and treatment of premalignant gastric lesions related to Helicobacter pylori infection. *Helicobacter* 2007;12:1–15
19 Nishi Y, Isomoto H, Uotani S, et al. Enhanced production of leptin in gastric fundic mucosa with Helicobacter pylori infection. *World J Gastroenterol* 2005;11:695–9
20 Azuma T, Suto H, Ito Y, et al. Gastric leptin and Helicobacter pylori infection. *Gut* 2001;49:324–9

21 Dixon MF, Genta RM, Yardley JH, et al. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996;20:1161–81

22 Dixon MF. Gastrointestinal epithelial neoplasia: Vienna revisited. *Gut* 2002;51:130–1

23 de Vries AC, Haringsma J, de Vries RA, et al. The use of clinical, histological and serological parameters to predict the intragastric extent of intestinal metaplasia: a recommendation for routine practice. *Gastointest Endosc* 2009;70:18–25

24 Frederich R, Hu S, Raymond N, et al. Leptin in anorexia nervosa and bulimia nervosa: importance of assay technique and method of interpretation. *J Lab Clin Med* 2002;139:72–9

25 Royston P, Altman DG, Sauerbrei W. Dichotomizing continuous predictors in multiple regression: a bad idea. *Stat Med* 2006;25:127–41

26 Little RJA, Rubin DB, eds. Statistical Analysis with Missing Data. New York: John Wiley, 2002

27 Youden WJ. Index rating for diagnostic tests. *Cancer* 1950;3:32–5

28 Sauerbruch T, Schreiber MA, Schussler P, et al. Endoscopy in the diagnosis of gastritis. Diagnostic value of endoscopic criteria in relation to histological diagnosis. *Endoscopy* 1984;16:101–4

29 Ley C, Mohar A, Guarner J, et al. Screening markers for chronic atrophic gastritis in Chiapas, Mexico. *Cancer Epidemiol Biomarkers Prev* 2001;10:107–12

30 Westerveld BD, Pals G, Lamers CB, et al. Clinical significance of pepsinogen A isozymogens, serum pepsinogen A and C levels, and serum gastrin levels. *Cancer* 1987;59:952–8

31 Kuipers EJ. In through the out door: serology for atrophic gastritis. *Eur J Gastroenterol Hepatol* 2003;15:877–9

32 Kendall BJ, Macdonald GA, Hayward NK, et al. Leptin and the risk of Barrett's oesophagus. *Gut* 2008;57:448–54

33 Saad MF, Damani S, Gingerich RL, et al. Sexual dimorphism in plasma leptin concentration. *J Clin Endocrinol Metab* 1997;82:579–84

34 Considine RV. Regulation of leptin production. *Rev Endocr Metab Disord* 2001;2:357–63

35 Jun DW, Lee OY, Lee YY, et al. Correlation between gastrointestinal symptoms and gastric leptin and ghrelin expression in patients with gastritis. *Dig Dis Sci* 2007;52:2866–72

36 Zhao L, Shen ZX, Luo HS, et al. Possible involvement of leptin and leptin receptor in developing gastric adenocarcinoma. *World J Gastroenterol* 2005;11:7666–70

37 Nwokolo CU, Freshwater DA, O'Hare P, et al. Plasma ghrelin following cure of Helicobacter pylori. *Gut* 2003;52:637–40

38 de Vries AC, van Grieken NC, Looman CW, et al. Gastric cancer risk in patients with premalignant gastric lesions: a nationwide cohort study in the Netherlands. *Gastroenterology* 2008;134:945–52

39 Cassaro M, Rugge M, Gutierrez O, et al. Topographic patterns of intestinal metaplasia and gastric cancer. *Am J Gastroenterol* 2000;95:1431–8

40 Sipponen P, Ranta P, Helske T, et al. Serum levels of amidated gastrin-17 and pepsinogen I in atrophic gastritis: an observational case-control study. *Scand J Gastroenterol* 2002;37:785–91

41 Kuipers EJ, Uyterlinde AM, Pena AS, et al. Long-term sequelae of Helicobacter pylori gastritis. *Lancet* 1995;345:1525–8

# Chapter 6

## Narrow band imaging for the detection of gastric intestinal metaplasia during surveillance endoscopy

Lisette G. Capelle[1], Jelle Haringsma[1], Annemarie C. de Vries[1], Ewout W. Steyerberg[2], Katharina Biermann[3], Herman van Dekken[3], Ernst J. Kuipers[1,4]

Departments of [1]Gastroenterology and Hepatology, [2]Public Health, [3]Pathology, and [4]Internal Medicine, Erasmus MC University Medical Center, Rotterdam, the Netherlands

*Digestive Diseases and Sciences; in press*

## ABSTRACT

*Background:* Surveillance of premalignant gastric lesions relies mainly on random biopsy sampling. Narrow band imaging (NBI) may enhance the accuracy of endoscopic surveillance of intestinal metaplasia (IM) and dysplasia. We aimed to compare the yield of NBI to white light endoscopy (WLE) in the surveillance of patients with IM and dysplasia.

*Methods:* Patients with previously identified gastric IM or dysplasia underwent a surveillance endoscopy. Both WLE and NBI were performed in all patients during a single procedure. The sensitivity of WLE and NBI for the detection of premalignant lesions was calculated by correlating endoscopic findings to histological diagnosis.

*Results:* Forty-three patients (28/15 MF, mean age 59 yrs) were included. IM was diagnosed in 27 patients; 20 were detected by NBI and WLE, 4 solely by NBI and 3 by random biopsies only. Dysplasia was detected in 7 patients by WLE and NBI and in 2 patients by random biopsies only. Sixty-eight endoscopically detected lesions contained IM; 47 were detected by WLE and NBI, 21 by NBI only. Nine endoscopically detected lesions demonstrated dysplasia; 8 were detected by WLE and NBI, 1 was detected by NBI only. The sensitivity, specificity, positive and negative predictive value for detection of premalignant lesions were 71%, 58%, 65% and 65% for NBI and 51%, 67%, 62% and 55% for WLE.

*Conclusions:* NBI considerably increases the diagnostic yield of the detection of advanced premalignant gastric lesions compared to routine WLE. NBI therefore seems superior to WLE in the surveillance of patients with these advanced gastric lesions.

## INTRODUCTION

*Helicobacter pylori* is an important risk factor for gastric cancer due to the fact that it causes chronic inflammation of the gastric mucosa in virtually all infected patients. In the multi-step model of gastric carcinogenesis, this chronic inflammation may slowly progress through the pre-malignant stages of atrophic gastritis, intestinal metaplasia and dysplasia to gastric adenocarcinoma.[1] We have previously shown that the actual cancer risk for patients with any of the pre-malignant conditions of the stomach is very similar to the cancer risk in patients with a Barrett's oesophagus or in those after removal of colonic adenoma.[2] Surveillance of these pre-malignant lesions could lead to early detection of patients with disease progression, and thus to early intervention aiming at cancer prevention and improved survival of these patients. However, recent investigation has demonstrated that current surveillance of pre-malignant gastric lesions is at great discrepancy with the substantial gastric cancer risk of these lesions.[2] Furthermore, studies have shown that a considerable proportion of patients with dysplastic lesions are being missed in current routine gastroenterology practice.[3]

The golden standard for diagnosing these gastric lesions is histology of biopsy specimens. The major shortcoming of this approach is the fact that pre-malignant lesions may occur multifocally, and may thus be missed on random biopsy sampling. Although image quality of standard endoscopes has improved dramatically over the past decades, endoscopic evaluation of the condition of the gastric mucosa still correlates poorly with histological findings.[4-7] Therefore, a diagnosis of pre-malignant gastric lesions remains dependent on random biopsy sampling during conventional gastroscopy.

Several new imaging techniques to overcome limitations of conventional white light endoscopy (WLE) have been developed over the last decades. A promising technique is narrow-band imaging (NBI). The principle of this new technique is based on modification of the spectral characteristics of the optical filter in the light source, which leads to improved visibility of mucosal structures. With use of different narrow-band filters in combination with image magnification, mucosal structures can be very clearly demonstrated, among others resulting in increased contrast between surface and vascular pattern.[8]

Several studies described the diagnostic accuracy of NBI in detecting gastrointestinal lesions.[8, 9] Based on these results, one might expect that the use of this technique for targeted biopsy sampling can increase the diagnostic yield of endoscopy for primary detection of pre-malignant gastric lesions. However, the additional value of NBI in the surveillance of patients with pre-malignant gastric lesions is yet unclear and requires further investigation.

Therefore the aim of this study was to compare the yield of NBI over conventional white light endoscopy (WLE) in the surveillance of patients with intestinal metaplasia and dysplasia, using histology as reference value.

## METHODS

This single center, prospective study was carried out in the Erasmus MC in Rotterdam. The study protocol was approved by the local Institutional Review Board. All patients provided informed consent.

### *Patient selection and endoscopic procedure*

Patients with previously identified intestinal metaplasia or dysplasia of the gastric mucosa underwent a surveillance endoscopy. Patients with coagulopathy uncorrected at the time of endoscopy or a thrombocytopenia (< 50 x $10^9$ / l) were excluded. After informed consent, both WLE and NBI were performed in all patients by a single endoscopist specialized in NBI and WLE endoscopy of the gastric mucosa during a single procedure with a GIF180 endoscope (Olympus Optical, Hamburg, Germany). The procedure started with conventional white light endoscopy. During endoscopy, all suspicious antral and angular gastric lesions were photographed, videotaped, and documented on a specially designed scoring sheet in terms of size (cm) and morphology (according to the Paris classification). During the same setting, the stomach was carefully observed using the NBI system. Again, all suspicious antral and angular gastric lesions were photographed, videotaped, and documented on the specially designed scoring sheet. NBI suspicious lesions for intestinal metaplasia were defined as bluish-whitish areas with an irregular mucosal pattern, a complete loss of architectural and mucosal pattern was suspicious for dysplasia. At least one targeted biopsy was taken from all endoscopic lesions suspected for intestinal metaplasia or dysplasia by NBI or WLE. Furthermore, 4 random biopsies were obtained; 2 from the antrum and 2 from the angulus.

### *Histological assessment*

Biopsy specimens obtained from the stomach were fixed in buffered formalin and embedded in paraffin. The specimens were sectioned at 4 µm thickness, and stained by haematoxylin and eosin. An expert pathologist specialized in GI pathology reviewed the sections and was blinded to endoscopic and clinical findings. Inflammation, atrophy, metaplasia and dysplasia were classified according to the updated Sydney System and revised Vienna classification.[10-12]

### *Statistical analysis*

The number of patients with intestinal metaplasia or dysplasia detected by NBI and WLE was evaluated. Furthermore, the number of endoscopically detected lesions which were suspected for intestinal metaplasia or dysplasia by NBI and/or WLE were evaluated. These endoscopically suspected lesions were considered as the unit of analysis in this evaluation, even

though some patients had more than 1 lesion. For the random biopsies, the overall diagnosis of the antrum biopsies and the overall diagnosis of the angulus biopsies were considered as unsuspected lesions by NBI or WLE. Each endoscopically suspected lesion (identified by NBI and WLE) or unsuspected lesion (random biopsy) was considered as an independent observation for statistical purposes. For each patient and each lesion only the most severe pre-malignant grading was evaluated. For instance, patients with intestinal metaplasia and with a concomitant diagnosis of dysplasia were classified as having dysplasia.

Sensitivity, specificity, positive and negative predictive values for the prediction of intestinal metaplasia and dysplasia for NBI and WLE were calculated using histology as reference value. Differences between NBI and WLE were assessed by using McNemar's test and by analyzing receiver operating characteristic (ROC) curves. In addition, a bootstrap resampling model (using c-stat) was performed to calculate the difference between the discriminatory power of both techniques.[13, 14] The data were submitted for statistical testing using the Statistical Package for the Social Sciences (SPSS), version 11.0.

## RESULTS

From May 2007 until December 2008, 43 patients (28 males, 15 females) with a mean age of 58.7 years (range 34 to 75 years) were included. Of these patients, 32 (74%) patients had a previous diagnosis of intestinal metaplasia and 11 (26%) patients had a previous diagnosis of dysplasia. The majority of patients (88%) were of Dutch descent. The baseline characteristics



**Table 1.** Baseline characteristics of patient population

| | Intestinal metaplasia n=32 (%) | Dysplasia* n= 11 (%) |
|---|---|---|
| Age (mean) (SD) | 57.5 (10.7) | 63.1 (9.8) |
| Sex | | |
| - Male | 20 (62) | 8 (73) |
| - Female | 12 (37) | 3 (27) |
| Ethnicity | | |
| - Caucasian | 29 (91) | 9 (82) |
| - Non-caucasian | 3 (9) | 2 (18) |
| Smoking | | |
| - Non- smoker | 19 (59) | 3 (27) |
| - Current smoker | 11 (34) | 3 (27) |
| - Former smoker | 2 (6) | 4 (36)** |
| Alcohol | | |
| - Non-drinker | 17 (53) | 5 (45) |
| - Current drinker | 15 (47) | 5 (45)** |
| Medication use | | |
| - PPI | 15 (47) | 4 (36) |
| - NSAIDs | 3 (9) | 2 (18) |
| *H. pylori* eradication therapy | 9 (28) | 5 (45) |

*Dysplasia; low grade dysplasia n=10; high grade dysplasia n=1; ** one patient refused to answer questions concerning smoking and drinking habits

of the patients are presented in Table 1. The mean interval between initial diagnosis and surveillance endoscopy was 2.0 years (range 0.8-21.1yrs) for patients with intestinal metaplasia and 1.9 (range 0.2-5.2 yrs) for patients with dysplasia.

### *Per patient analysis*

Of the 43 patients that were included, 27 (63%) demonstrated intestinal metaplasia at surveillance endoscopy and 9 (21%) patients demonstrated dysplasia (low grade dysplasia n=6; high grade dyplasia n=3) (Table 2, Figure 1 & 2). In the remaining 7 (16%) patients no diagnosis of intestinal metaplasia or dysplasia was confirmed at surveillance endoscopy. Baseline endoscopy had shown intestinal metaplasia in antrum and corpus mucosa in 5 (12%) and 2 (4%) of these patients respectively.

**Table 2.** Patients with histologically confirmed diagnosis of intestinal metaplasia (IM) or dysplasia (DYS)

| | *Histologically confirmed* | | |
|---|---|---|---|
| | IM | DYS | Total |
| | N=27 | N=9 | N=36* |
| *Detected by* | | | |
| - WLE & NBI | 20 | 7 | 27 |
| - NBI | 4 | - | 4 |
| - WLE | - | - | - |
| - Random biopsies | 3 | 2 | 5 |

* In the remaining 7 patients no diagnosis of intestinal metaplasia or dysplasia was confirmed

Of the 27 intestinal metaplasia patients, 20 (74%) patients were detected by both WLE and NBI, whereas 4 (15%) patients were detected solely by NBI. The remaining three (11%) patients were detected by random biopsy sampling only. Of the 9 dysplasia patients, 7 (78%) (low grade dysplasia n=4 and high grade dysplasia n=3) patients were detected by both WLE

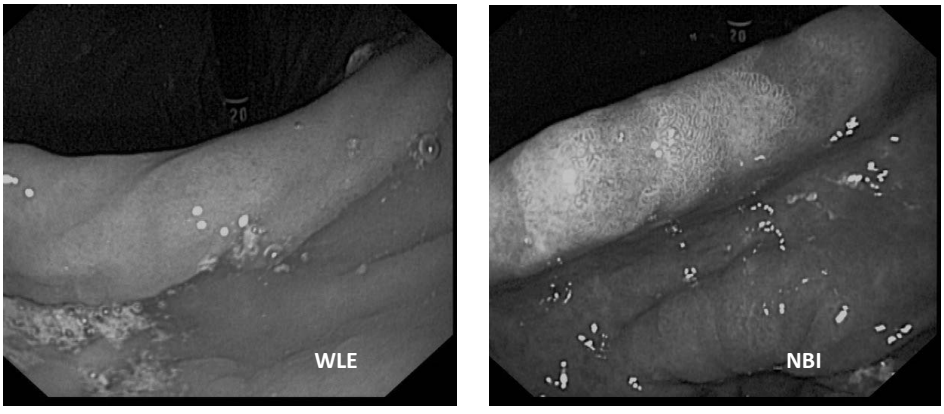


**Figure 1.** WLE image and NBI image of intestinal metaplasia at the angulus

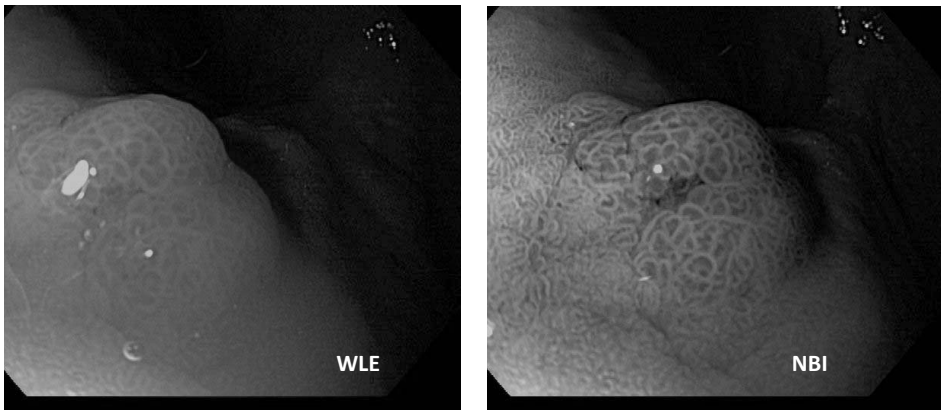


**Figure 2.** WLE image and NBI image of dysplasia at the angulus

and NBI and not by random biopsies. In the remaining two (22%) patients, random biopsies demonstrated foci with low grade dysplasia. NBI and WLE detected marked intestinal metaplasia but no dysplasia in these 2 patients.

### *Per lesion analysis*

In total, 121 lesions in the gastric mucosa were endoscopically suspected for intestinal metaplasia or dysplasia after WLE and NBI (Table 3). Eighty-six (71%) of these endoscopic lesions were suspected for intestinal metaplasia or dysplasia by both WLE and NBI. Two (2%) were only suspected by WLE, and 33 (27%) were only suspected by NBI.

**Table 3.** Endoscopically suspected lesions and a histologically confirmed diagnosis of intestinal metaplasia (IM) or dysplasia (DYS)

| | *Histologically confirmed* | | | |
|---|---|---|---|---|
| | IM (n=68) | DYS (n=9) | No IM/DYS (n=44) | Total (n=121) |
| *Suspected lesions by* | | | | |
| - WLE & NBI | 47 | 8 | 31 | 86 |
| - NBI | 21 | 1 | 11 | 33 |
| - WLE | - | - | 2 | 2 |

Seventy-seven (64%) of these 121 endoscopically suspected lesions had a histopathological diagnosis of intestinal metaplasia (n=68) or dysplasia (n=9). For intestinal metaplasia (n=68), 47 (69%) endoscopical lesions were detected both by WLE and NBI, the remaining 21 (30%) lesions were solely detected by NBI. For dysplasia (n=9), 8 (89%) endoscopic lesions (low grade dysplasia n=5 and high grade dysplasia n=3) were detected by WLE and NBI, whereas one (11%) lesion with low grade dysplasia was detected by NBI only.

Although all 121 endoscopic lesions were suspected for intestinal metaplasia or dysplasia by NBI or WLE, forty-four (36%) of these 121 suspected lesions did not show intestinal

metaplasia or dysplasia when histologically assessed. Thirty-one (70%) of these false positive lesions were suspected both by WLE and NBI, two (5%) were suspected only by WLE, and 11 (25%) were suspected by NBI only.

The overall diagnosis of the random biopsies resulted in 27 additional endoscopically suspected lesions which showed intestinal metaplasia and 4 lesions which showed low grade dysplasia. All these regions had not been suspected endoscopically by NBI or WLE.

Based on these results, the sensitivity, specificity, positive and negative predictive value of WLE endoscopy were 51%, 67%, 62% and 55% respectively. For NBI, the sensitivity, specificity, positive predictive value and negative predictive value were 71%, 58%, 65% and 65%, respectively. Specificity was marginally higher for WLE ($p=0.04$), whereas sensitivity was considerably lower for WLE than for NBI ($p<0.001$). In addition, according to bootstrap resampling, NBI was superior in detecting intestinal metaplasia and dysplasia versus normal mucosa than WLE ($p=0.03$).

## DISCUSSION

This study provides evidence that NBI yields more accurate results in the surveillance of patients with intestinal metaplasia and dysplasia than conventional WLE. Firstly, we demonstrated that 15% of the patients with intestinal metaplasia at surveillance endoscopy were solely detected by NBI. Secondly, considerably more endoscopically detected lesions with intestinal metaplasia were detected by NBI compared to WLE. And thirdly, the sensitivity for the detection of advanced precursor lesions increased by 20% to 71% for NBI.

Similar to our observations, previous studies demonstrated promising results for NBI for the detection of pre-neoplastic lesions in the gastrointestinal tract, in particular for colon and esophagus.[15] The additional value of NBI in the detection of gastric pre-malignant lesions remained less clear, especially in countries with a low gastric cancer incidence.

A Japanese study described a sensitivity and specificity of 89% and 93% respectively for the detection of gastric intestinal metaplasia with NBI endoscopy.[8] This high accuracy compared to our findings is probably explained by training differences that exist between Japanese and Western gastroenterologists. Due to the high gastric cancer incidence, Japanese endoscopists are trained to scrutinize gastric mucosal areas which are compatible with atrophy and early cancer. Moreover, considerably more time is spent on a thorough mucosal examination, than in Western countries.[16] Another possible explanation for the high sensitivity and specificity found in the previous study was the use NBI in combination magnification endoscopy.[8] In Japan, it has been demonstrated that magnification endoscopy can accurately detect gastric cancer during routine endoscopy.[17-19] However, our study shows that in Western countries with an overall low gastric cancer incidence, even without adding magnification, NBI endos-

copy is of additional value for the detection of pre-malignant gastric lesions, in particular in a surveillance setting.

Currently, the diagnosis of intestinal metaplasia and dysplasia is based on histological evaluation of biopsy specimens. Since endoscopic diagnosis of pre-malignant lesions shows high interobserver variability and has poor correlation to histological diagnosis, numerous other endoscopic techniques have been developed to overcome these limitations in the last decades.

Similar to NBI, the use of auto-fluorescence endoscopy demonstrated a high correlation between Barrett's esophagus and histological diagnosis. However, the correlation between gastric cancer and this imaging technique still remains controversial.[20-22] For chromoendoscopy, a previous study demonstrated a facilitated detection of early gastric cancer in hereditary diffuse gastric cancer.[23] Moreover, compared to auto-fluorescence endoscopy, the equipment necessary for chromoendoscopy is widely available and the technique is often quick and inexpensive. For some new staining techniques however, safety remains questionable.[24]

Confocal endomicroscopy is a newly developed endoscopic technique that produces 1000-fold magnification cross-sectional images. This new technique can accurately predict the presence of early cancer in targeted areas, and a recently published gastric pit-pattern classification for the prediction for gastritis and atrophy showed a high correlation with histology.[25, 26] Nevertheless, confocal endomicroscopy is not able to completely replace histology and interobserver and intraobserver agreement for this pit-pattern classification remains unknown. Furthermore, the technique is too elaborate to be used for assessment of the complete gastric mucosa.

Compared to these new techniques, magnification endoscopy demonstrated best sensitivities and specificities for a diagnosis of atrophic gastritis or gastric cancer. However, similar to NBI, most of these previous studies were of Japanese origin and mostly included low numbers of patients.[17, 18, 27, 28] In addition, despite the promising results of magnification endoscopy, uniform classification criteria of this technique have till this date not been confirmed in large controlled trials in Western or Eastern countries.

Since our study shows that NBI has a low specificity and suboptimal sensitivity for the detection of preneoplastic gastric lesions, a combination of both NBI as well as magnification is likely to provide the best alternative with current endoscopical practice. Previous studies already demonstrated a high correlation between microvascular patterns found with NBI in combination with magnification, and a diagnosis of gastric cancer.[9, 29] Therefore, further research in a prospective study design is necessary to evaluate whether NBI in combination with magnification also yields adequate results for the detection and surveillance of patients with intestinal metaplasia or dysplasia in Western countries.

Previous studies demonstrated that surveillance of patients with pre-malignant gastric lesions should preferably be limited to patients at high risk of gastric cancer.[2] A risk score based

on histology only (OLGIM staging system) or a broader risk classification including several clinical and laboratory parameters have been described.[30, 31] For either method, adequate biopsy sampling at baseline is essential. In this study we show that NBI has the potential to increase and optimalize the yield of biopsies with intestinal metaplasia. However, although NBI shows an improved sensitivity for the detection of premalignant gastric lesions over WLE, random biopsy sampling is still necessary in the surveillance of patients with pre-malignant gastric lesions. This is illustrated by the fact that three patients with intestinal metaplasia and two patients with dysplasia were not detected after WLE and NBI endoscopy and were only diagnosed with these pre-malignant lesions after histological evaluation of the random biopsies. Therefore, targeted and random biopsies seem essential for accurate surveillance of patients with pre-malignant gastric lesions. A further study in our department concerning the use of random and targeted biopsies, demonstrated that 9 random biopsies from cardia, corpus, in particular along lesser curvature, angulus and antrum are required for optimal detection of pre-malignant gastric lesions in a population at low gastric cancer risk.[32] However, similar to this previous study, in a small percentage of patients of our study, intestinal metaplasia was not confirmed during surveillance endoscopy.[32] Since these patients all underwent endoscopy with extensive biopsy sampling, we assume that the majority, if not all of these patients had a patchy and limited extent of metaplasia and thus a low gastric cancer risk.

Some limitations of this study warrant consideration. Firstly, the endoscopic procedure of WLE and NBI was performed by the same endoscopist. Therefore, detection of intestinal metaplasia and dysplasia by NBI could possibly be biased by the previous white light observations, resulting in an overestimation of the detection rate of NBI. Secondly, only recently it has been demonstrated that the severity and extent of atrophic gastritis and intestinal metaplasia are adequate predictors of gastric cancer risk. [31, 33] Antrum and angulus were selected in this study because these are the regions of particular interest with the highest prevalence of intestinal metaplasia. Nevertheless, the protocol used in this study is also applicable to the proximal part of the gastric mucosa. A large further study is necessary to confirm that NBI in combination with random biopsy sampling may accurately detect extensive intestinal metaplasia in patients with pre-malignant gastric lesions.

Thirdly, although NBI showed an increased detection rate for intestinal metaplasia and dysplasia, it was also related to a higher rate of false positivity than WLE (Table 3). This higher rate does however not imply increasing costs for surveillance, as the decision to embark on surveillance remains dependent on confirmation of endoscopic findings by histology.

In conclusion, NBI considerably increases the diagnostic yield of the detection of advanced premalignant gastric lesions compared to routine WLE. Therefore, NBI seems superior to WLE in the surveillance of patients with these advanced lesions of the gastric mucosa.

## REFERENCES


1 Correa P. Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 1992;52:6735-40.

2 de Vries AC, van Grieken NC, Looman CW, *et al.* Gastric cancer risk in patients with premalignant gastric lesions: a nationwide cohort study in the Netherlands. *Gastroenterology* 2008;134:945-52.

3 Lassen A, Hallas J, de Muckadell OB. The risk of missed gastroesophageal cancer diagnoses in users and nonusers of antisecretory medication. *Gastroenterology* 2005;129:1179-86.

4 Sauerbruch T, Schreiber MA, Schussler P, *et al.* Endoscopy in the diagnosis of gastritis. Diagnostic value of endoscopic criteria in relation to histological diagnosis. *Endoscopy* 1984;16:101-4.

5 Redeen S, Petersson F, Jonsson KA, *et al.* Relationship of gastroscopic features to histological findings in gastritis and Helicobacter pylori infection in a general population sample. *Endoscopy* 2003;35:946-50.

6 Lin BR, Shun CT, Wang TH, *et al.* Endoscopic diagnosis of intestinal metaplasia of stomach--accuracy judged by histology. *Hepatogastroenterology* 1999;46:162-6.

7 Meshkinpour H, Orlando RA, Arguello JF, *et al.* Significance of endoscopically visible blood vessels as an index of atrophic gastritis. *Am J Gastroenterol* 1979;71:376-9.

8 Uedo N, Ishihara R, Iishi H, *et al.* A new method of diagnosing gastric intestinal metaplasia: narrow-band imaging with magnifying endoscopy. *Endoscopy* 2006;38:819-24.

9 Nakayoshi T, Tajiri H, Matsuda K, *et al.* Magnifying endoscopy combined with narrow band imaging system for early gastric cancer: correlation of vascular pattern with histopathology (including video). *Endoscopy* 2004;36:1080-4.

10 Dixon MF, Genta RM, Yardley JH, *et al.* Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996;20:1161-81.

11 Dixon MF. Gastrointestinal epithelial neoplasia: Vienna revisited. *Gut* 2002;51:130-1.

12 Schlemper RJ, Kato Y, Stolte M. Diagnostic criteria for gastrointestinal carcinomas in Japan and Western countries: proposal for a new classification system of gastrointestinal epithelial neoplasia. *J Gastroenterol Hepatol* 2000;15 Suppl:G49-57.

13 Steyerberg EW. *Clinical Prediction Model: A Practical Approach to Development, Validation and Updating*. New York: Springer Edition 2009.

14 Altman DG. ROC curves and confidence intervals: getting them right. *Heart* 2000;83:236.

15 East JE, Tan EK, Bergman JJ, *et al.* Meta-analysis: narrow band imaging for lesion characterization in the colon, oesophagus, duodenal ampulla and lung. *Aliment Pharmacol Ther* 2008;28:854-67.

16 de Vries AC, Haringsma J, Kuipers EJ. The detection, surveillance and treatment of premalignant gastric lesions related to Helicobacter pylori infection. *Helicobacter* 2007;12:1-15.

17 Ohashi A, Niwa Y, Ohmiya N, *et al.* Quantitative analysis of the microvascular architecture observed on magnification endoscopy in cancerous and benign gastric lesions. *Endoscopy* 2005;37:1215-9.

18 Tajiri H, Doi T, Endo H, *et al.* Routine endoscopy using a magnifying endoscope for gastric cancer diagnosis. *Endoscopy* 2002;34:772-7.

19 Otsuka Y, Niwa Y, Ohmiya N, *et al.* Usefulness of magnifying endoscopy in the diagnosis of early gastric cancer. *Endoscopy* 2004;36:165-9.

20 Ortner MA, Ebert B, Hein E, *et al.* Time gated fluorescence spectroscopy in Barrett's oesophagus. *Gut* 2003;52:28-33.

21 Kato M, Kaise M, Yonezawa J, *et al.* Autofluorescence endoscopy versus conventional white light endoscopy for the detection of superficial gastric neoplasia: a prospective comparative study. *Endoscopy* 2007;39:937-41.
22 Mayinger B, Jordan M, Horbach T, *et al.* Evaluation of in vivo endoscopic autofluorescence spectroscopy in gastric cancer. *Gastrointest Endosc* 2004;59:191-8.
23 Shaw D, Blair V, Framp A, *et al.* Chromoendoscopic surveillance in hereditary diffuse gastric cancer: an alternative to prophylactic gastrectomy? *Gut* 2005;54:461-8.
24 Mouzyka S, Fedoseeva A. Chromoendoscopy with hematoxylin in the classification of gastric lesions. *Gastric Cancer* 2008;11:15-21; discussion -2.
25 Zhang JN, Li YQ, Zhao YA, *et al.* Classification of gastric pit patterns by confocal endomicroscopy. *Gastrointest Endosc* 2008;67:843-53.
26 Dunbar K, Canto M. Confocal endomicroscopy. *Curr Opin Gastroenterol* 2008;24:631-7.
27 Anagnostopoulos GK, Yao K, Kaye P, *et al.* High-resolution magnification endoscopy can reliably identify normal gastric mucosa, Helicobacter pylori-associated gastritis, and gastric atrophy. *Endoscopy* 2007;39:202-7.
28 Tanaka K, Toyoda H, Kadowaki S, *et al.* Features of early gastric cancer and gastric adenoma by enhanced-magnification endoscopy. *J Gastroenterol* 2006;41:332-8.
29 Kaise M, Kato M, Urashima M, *et al.* Magnifying endoscopy combined with narrow-band imaging for differential diagnosis of superficial depressed gastric lesions. *Endoscopy* 2009;41:310-5.
30 Capelle LG, de Vries AC, Haringsma J, *et al.* The staging of gastritis with the OLGA system using intestinal metaplasia as accurate alternative for atrophic gastritis. *Gastrointest Endosc* 2010;Accepted.
31 de Vries AC, Haringsma J, de Vries RA, *et al.* The use of clinical, histologic, and serologic parameters to predict the intragastric extent of intestinal metaplasia: a recommendation for routine practice. *Gastrointest Endosc* 2009;70:18-25.
32 de Vries AC, Haringsma J, de Vries RA, *et al.* The yield of endoscopic surveillance of pre-malignant gastric lesions: optimalization of biopsy strategies. *submitted* 2009.
33 Rugge M, Genta RM. Staging gastritis: an international proposal. *Gastroenterology* 2005;129:1807-8.

# Chapter 7

## The staging of gastritis with the OLGA system using intestinal metaplasia as accurate alternative for atrophic gastritis

Lisette G. Capelle[1], Annemarie C. de Vries[1], Jelle Haringsma[1], Frank ter Borg[2], Richard A. de Vries[3], Marco J. Bruno[1], Herman van Dekken[4], Jos Meijer[5], Nicole C. T. van Grieken[6], and Ernst. J. Kuipers[1,7]

[1]Departments of Gastroenterology and Hepatology, [4]Pathology, and [7]Internal Medicine, Erasmus MC University Medical Center, Rotterdam, [2]Department of Hepato-gastroenterology, Deventer Hospital, Deventer, [3]Department of Internal Medicine, University Medical Center, Groningen, [5]Department of Pathology, Rijnstate Hospital, Arnhem, [6]Department of Pathology, VU University Medical Center, *Amsterdam.*

*Gastrointestinal Endoscopy; in press*

## ABSTRACT

*Background:* The OLGA (operative link on gastritis assessment) staging system is based on severity of atrophic gastritis (AG). AG remains a difficult histopathologic diagnosis with low interobserver agreement, whereas intestinal metaplasia (IM) is associated with high interobserver agreement.

*Objective:* The aim of this study was to evaluate whether a staging system based on IM is preferable to estimate gastric cancer risk.

*Design and Setting:* Prospective multicenter study.

*Patients:* A total of 125 patients previously diagnosed with gastric IM or dysplasia.

*Interventions:* Surveillance endoscopy with extensive biopsy sampling.

*Main Outcome Measurements:* Three pathologists graded biopsy specimens according to the Sydney classification. Interobserver agreement was analyzed by kappa statistics. In the OLGA, AG was replaced by IM, creating the OLGIM.

*Results:* Interobserver agreement was fair for dysplasia ($\kappa = 0.4$), substantial for AG ($\kappa = 0.6$), almost perfect for IM ($\kappa = 0.9$), and improved for all stages of OLGIM compared with OLGA. Overall, 84 (67%) and 79 (63%) patients were classified as stage I-IV according to OLGA and OLGIM, respectively. Of the dysplasia patients, 5 (71%) and 6 (86%) clustered in stage III-IV of OLGA and OLGIM, respectively.

*Limitation:* Prospective studies should confirm the correlation between gastric cancer risk and OLGIM stages.

*Conclusion:* Replacement of AG by IM in the staging of gastritis considerably increases interobserver agreement. The correlation with the severity of gastritis remains at least as strong. Therefore, the OLGIM may be preferred over the OLGA for the prediction of gastric cancer risk in patients with premalignant lesions.

## INTRODUCTION

The presence of atrophic gastritis (AG), intestinal metaplasia (IM), and dysplasia of the gastric mucosa are important risk factors for the intestinal type of gastric cancer.[1,2] Surveillance of patients with these lesions may therefore result in early detection and improved prognosis.[3] However, an earlier study demonstrated that within a Western population, the progression rate to gastric cancer within 10 years was high for patients with dysplasia, but only 0.8% and 1.8% for patients with AG and IM, respectively.[3] This indicates that surveillance endoscopy is highly recommended for patients with dysplasia, but not indicated for all patients with AG and IM, and should preferably be limited to patients at high gastric cancer risk. However, up to now no guidelines are available on endoscopic surveillance of patients with premalignant gastric lesions.

Although several histologic classifications have been proposed for the classification of premalignant gastric lesions, clinical implications based on these histologic systems are lacking.[4,5] Consequently, histologic subclassification of premalignant gastric lesions is often omitted in clinical practice. Only recently, a histologic classification system was proposed to grade gastritis into stages with corresponding cancer risks in individual patients: the operative link on gastritis assessment (OLGA).[6,7] Two validation studies reported that the OLGA provides clinically relevant information and, as a consequence, identifies a subpopulation of patients that are at high risk of gastric cancer and may benefit from surveillance.[8,9]

However, one potential shortcoming of the OLGA is the fact that its main parameter is the severity and the extent of AG. Studies have shown that the interobserver agreement for AG is low, even after the updated Sydney system provided visual analog scales for its evaluation.[4,10-12]

Intestinal metaplasia is defined as replacement of gastric columnar cells by cells of intestinal morphology and is characterized by the presence of mucin-containing goblet cells, Paneth cells, and absorptive cells.[13] These cells are easily distinguished in the gastric mucosa, because they are not present in healthy gastric mucosa. Therefore, IM is associated with a high interobserver agreement.[4,14] A histologic staging system based on IM might yield additional and more accurate results for the identification of a subpopulation of patients at high gastric cancer risk. Therefore, the aim of the present study was to evaluate interobserver agreement for AG, IM, and dysplasia and to assess whether a staging system based on IM instead of AG may be preferred to estimate gastric cancer risk.

## METHODS

### *Patient selection*

We studied 2 groups of patients. The first group included patients with a previous diagnosis of gastric IM or dysplasia. For that purpose, we used the records between 1994 and 2009 of the histology registries of the participating hospitals (Deventer Hospital, Deventer; Rijnstate Hospital, Arnhem; Erasmus Medical Center, Rotterdam, The Netherlands) to identify patients who were eligible for inclusion. In these registries with full coverage of all histopathological specimens, all biopsy specimens receive a diagnostic code, similar to the Systematized Nomenclature of Medicine classification of the College of American Pathologists.[15] This code consists of a term indicating the anatomic location, type of sample, and a morphologic term describing the finding. The diagnostic codes that were used to identify patients with IM or dysplasia were "intestinal metaplasia" or "dysplasia." Consecutive patients with a histologically confirmed diagnosis of IM or dysplasia of the gastric mucosa (index diagnosis) were invited to undergo a surveillance endoscopy between March 2006 and June 2007. The surveillance endoscopy was performed within 6 years after the initial diagnosis of IM. The baseline endoscopy had in all cases been performed on clinical grounds, usually because of upper GI symptoms. None of the patients had been enrolled in a surveillance program after the baseline endoscopy.

The second group included patients with gastric cancer. These were also selected from the same database, using the diagnostic codes "gastric carcinoma" and "gastric adenocarcinoma." Patients with a history of esophageal or gastric surgery were excluded. For the purpose of this study, biopsy specimens from the noncancerous antrum and corpus mucosa were studied after histologic confirmation of the diagnosis of cancer.

The study was approved by the Institutional Review Boards of the Erasmus Medical Center. All patients of the first group were included after informed consents. For the second group of patients, the informed consent procedure was waived, based on the fact that the study only anonymously assessed their archived histologic specimens.

### *Endoscopy*

All patients with a previous diagnosis of IM and dysplasia underwent a surveillance upper GI endoscopy by using a standard video endoscope (Olympus GIF-Q160; Olympus Optical Co., Tokyo, Japan). Surveillance endoscopy was performed to evaluate the severity and extent of premalignant gastric lesions. Therefore, extensive biopsy samples were obtained for histology from 12 standardized sites as described perviously[16]: 4 from the antrum, 4 from the corpus (2 from the lesser curvature, 2 from the greater curvature), 2 from the angulus, and 2

from the cardia. In case of endoscopically visible lesions, additional targeted biopsy samples were obtained.

### *Histology*

Three expert GI pathologists, who were blinded for the endoscopic findings, independently assessed all biopsy specimens of the surveillance endoscopy. The type and grade of the different stages of gastric preneoplastic changes were classified according to the updated Sydney system and scored as 0 (absent), 1 (mild), 2 (moderate), or 3 (marked) by using the Sydney system visual analog scale.[4] Dysplasia was assessed according to the revised Vienna classification.[4] and [5] On the basis of the standardized sites, the gastritis stage was assessed according to the OLGA (Table 1).[6] For the development of the IM staging system (operative link on gastric intestinal metaplasia assessment [OLGIM]), AG in the OLGA was replaced by IM (Table 2). AG and IM were scored in all biopsy specimens from antrum, angulus, and corpus lesser and greater curvature by using the visual analog scale of the updated Sydney classification (Fig. 1).[4] For a consensus diagnosis, the final diagnosis was based on the majority diagnosis, ie, at least 2 of 3 pathologists agreed, or a mean score in case 3 pathologists disagreed. Antrum

**Table 1.** The OLGA staging system [6]

| | | Corpus | | | |
|---|---|---|---|---|---|
| | Atrophy score | No atrophy (score 0) | Mild atrophy (score 1) | Moderate atrophy (score 2) | Severe atrophy (score 3) |
| Antrum | No atrophy (score 0) (including incisura angularis) | Stage 0 | Stage I | Stage II | Stage II |
| | Mild atrophy (score 1) (including incisura angularis) | Stage I | Stage I | Stage II | Stage III |
| | Moderate atrophy (score 2) (including incisura angularis) | Stage II | Stage II | Stage III | Stage IV |
| | Severe atrophy (score 3) (including incisura angularis) | Stage III | Stage III | Stage IV | Stage IV |

**Table 2.** Proposal for the OLGIM staging system

| | | Corpus | | | |
|---|---|---|---|---|---|
| | Intestinal metaplasia score | No intestinal metaplasia (score 0) | Mild intestinal metaplasia (score 1) | Moderate intestinal metaplasia (score 2) | Severe intestinal metaplasia (score 3) |
| Antrum | No intestinal metaplasia (score 0) (including incisura angularis) | Stage 0 | Stage I | Stage II | Stage II |
| | Mild intestinal metaplasia (score 1) (including incisura angularis) | Stage I | Stage I | Stage II | Stage III |
| | Moderate intestinal metaplasia (score 2) (including incisura angularis) | Stage II | Stage II | Stage III | Stage IV |
| | Severe intestinal metaplasia (score 3) (including incisura angularis) | Stage III | Stage III | Stage IV | Stage IV |

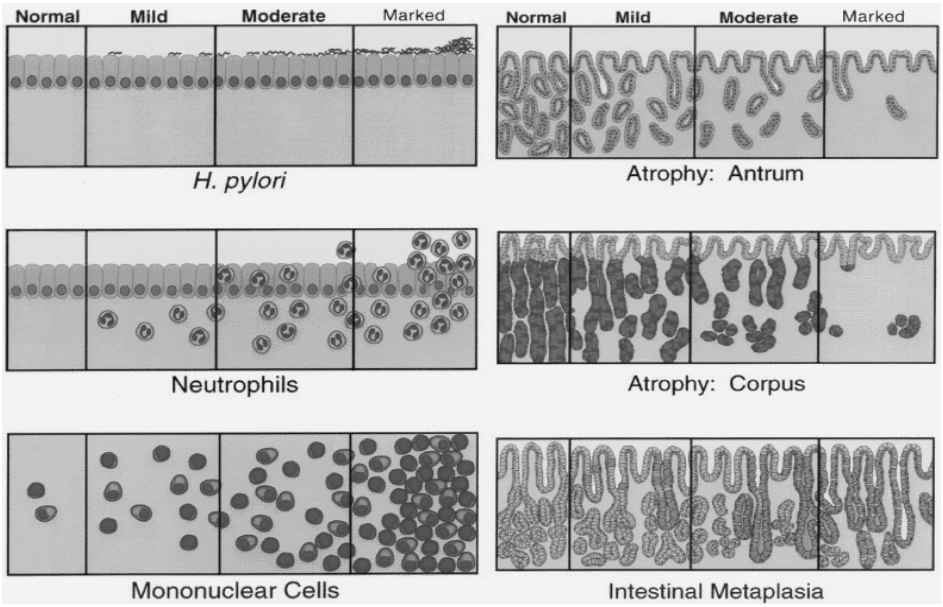


**Figure 1.** Visual analogue scale [4]
Atrophic gastritis and intestinal metaplasia were scored according to visual analogue scale of the updated Sydney classification as previously published by Dixon MF, *et al.* Am J Surg Pathol 1996; 20:1161-81. [4]

and angulus were considered together as representative of the distal (nonoxyntic) gastric mucosa (antrum score), and corpus greater and lesser curvature were considered together as representative of the oxyntic gastric mucosa (corpus score). Combining the antrum and corpus score for AG resulted in the OLGA gastritis stage score, and a combination of the IM scores resulted in the OLGIM staging score (Table 2).

For the gastric cancer cases, 1 expert GI pathologist assessed all biopsy specimens of patients with gastric cancer. The type and grade of atrophic gastritis and intestinal metaplasia were assessed according to the updated Sydney classification in biopsies from the non cancerous mucosa of the antrum and corpus mucosa. These scores were combined to evaluate OLGA and OLGIM staging in gastric cancer patients.

### *Statistical analysis*

Interobserver agreement was determined by using kappa statistics for multiple raters.[17] Kappa statistics are widely used mathematical coefficients adjusting for agreement by chance alone. Kappa values between 0 and 1 were categorized after Landis: 0 is no agreement, 0.01 to ≤0.20 is slight agreement, 0.21 to ≤0.40 is fair agreement, 0.41 to ≤0.60 is moderate agreement, 0.61 to ≤0.80 is substantial agreement, and 0.81 to ≤1.0 is almost perfect agreement.[18] Kappa statistics were evaluated for AG, IM, and dysplasia in the random and targeted biopsies

to assess the overall agreement. For the agreement per intragastric location, kappas were calculated for the presence of AG, IM, and dysplasia in the random biopsies. The stages 0-IV of the OLGA and the stages 0-IV of the OLGIM were evaluated for agreement, patient characteristics, patient distribution, and gastric cancer risk. Categoric variables were compared by using chi-square tests and the McNemar test. A 2-sided *P* value of <.05 was considered to be statistically significant.

## RESULTS

Overall, 204 patients were eligible for inclusion. Contact information was missing or wrong in 28 patients, and 51 patients refused to participate in this study. In total, 125 patients with a previous diagnosis of IM or dysplasia (69 male, 56 female) with a mean (±SD) age of 61 ± 11.7 years underwent surveillance endoscopy (Table 3). Ninety-eight patients (78%) were of Dutch origin, 53 patients (42%) had a previous history of *Helicobacter pylori* eradication, and 41 patients (33%) had a history of peptic ulcer disease. According to the index histologic findings, 63 patients (50%) had been diagnosed with IM and 62 (50%) with dysplasia (Table

**Table 3.** Baseline characteristics and OLGA and OLGIM staging of our study population

| | | OLGA staging | | | | | OLGIM staging | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total n=125 (%) | Stage 0 | Stage 1 | Stage 2 | Stage 3 | Stage 4 | Stage 0 | Stage 1 | Stage 2 | Stage 3 | Stage 4 |
| **N** | 125 | 41 | 24 | 30 | 22 | 8 | 46 | 22 | 28 | 20 | 9 |
| **Sex** | | | | | | | | | | | |
| - Male | 69 (55) | 26 | 11 | 15 | 14 | 3 | 27 | 11 | 14 | 14 | 3 |
| - Female | 56 (45) | 15 | 13 | 15 | 8 | 5 | 19 | 11 | 14 | 6 | 6 |
| **Age mean (SD)** | 61 (11.7) | 56.9 | 64.4 | 63.7 | 60.6 | 66.4 | 58.0 | 63.2 | 63.0 | 61.8 | 66.0 |
| **Ethnicity** | | | | | | | | | | | |
| - Caucasian | 98 (78) | 26 | 20 | 27 | 17 | 8 | 32 | 17 | 24 | 16 | 9 |
| - Non-Caucasian | 27 (22) | 15 | 4 | 3 | 5 | 0 | 14 | 5 | 4 | 4 | 0 |
| **Medication use** | | | | | | | | | | | |
| - PPI | 74 (59) | 25 | 15 | 14 | 13 | 7 | 28 | 13 | 12 | 13 | 8 |
| - NSAIDs | 22 (18) | 33 | 3 | 7 | 3 | 1 | 7 | 5 | 6 | 2 | 2 |
| ***H. pylori* eradication** | 53 (42) | 18 | 11 | 9 | 11 | 4 | 19 | 9 | 8 | 13 | 4 |
| **Index endoscopy** | | | | | | | | | | | |
| - IM | 63 (50) | 21 | 9 | 18 | 11 | 4 | 24 | 9 | 16 | 10 | 4 |
| - DYS | 62 (50) | 20 | 15 | 12 | 11 | 4 | 22 | 13 | 12 | 10 | 5 |
| **Surveillance endoscopy** | | | | | | | | | | | |
| - None | 33 (26) | 33 | 0 | 0 | 0 | 0 | 33 | 0 | 0 | 0 | 0 |
| - AG | 9 (7) | 2 | 6 | 0 | 1 | 0 | 9 | 0 | 0 | 0 | 0 |
| - IM | 76 (61) | 6 | 17 | 29 | 19 | 5 | 4 | 22 | 27 | 18 | 5 |
| - LGD | 5 (4) | 0 | 1 | 1 | 1 | 2 | 0 | 0 | 1 | 1 | 3 |
| - HGD | 2 (2) | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 |

IM: Intestinal metaplasia; DYS: dysplasia; AG: atrophic gastritis; LGD: low grade dysplasia; HGD: high grade dysplasia

3). At surveillance endoscopy, 9 patients (7%) were diagnosed with AG and 76 (61%) with IM as the most advanced lesion. Low-grade and high-grade dysplasia were diagnosed in 5 (4%) and 2 (2%) patients, respectively. In the remaining 33 patients (26%), no premalignant lesion was diagnosed, and 29 (89%) were diagnosed with chronic active gastritis.

The biopsy specimens of 30 patients with a diagnosis of gastric cancer were collected. After histologic revision, the biopsy specimens of 10 (33%) of these patients were excluded because either the antrum or the corpus specimens did not contain noncancerous tissue required for OLGA and OLGIM classification. The biopsy specimens of the 20 remaining gastric cancer patients (67%) were included for gastric cancer risk assessment.

### *Interobserver agreement*

Overall, agreement between 3 GI pathologists was moderate to substantial for AG ($\kappa = 0.6$) and almost perfect for IM ($\kappa = 0.9$) (Table 4). There was slight agreement for low-grade dysplasia and moderate agreement for high-grade dysplasia ($\kappa = 0.2$ and $\kappa = 0.5$, respectively). Table 4 demonstrates the agreement for the overall diagnosis, based on random and targeted biopsies together, as well as the agreement per intragastric localization based on random biopsies only. The agreement for antral and angular random biopsies for AG was moderate ($\kappa = 0.5$ and $\kappa = 0.6$, respectively), whereas agreement for IM for both intragastric localizations was almost perfect ($\kappa = 0.8$ and $\kappa = 0.9$, respectively). For corpus biopsies, overall agreement for AG was substantial to almost perfect for corpus greater curvature and corpus lesser curvature and was almost perfect for both localizations for IM (Table 4). Agreement for dysplasia varied from no or slight agreement for the antrum, angulus, cardia, and greater curvature biopsies of the corpus to fair agreement for the corpus lesser curvature. Table 5 demonstrates the agreement for the stages of OLGA and OLGIM. The overall agreement was fair for the OLGA and moderate for the OLGIM. Both the individual stages III and IV as well as their combination had an improved interobserver agreement in the OLGIM compared with the OLGA (Table 5).

**Table 4.** Interobserver agreement (kappa values) for the overall agreement and agreement per intragastric localisation

| | Overall* | Antrum# | Angulus# | Corpus greater curvature# | Corpus lesser curvature# | Cardia# |
|---|---|---|---|---|---|---|
| AG | 0.64 | 0.47 | 0.59 | 0.77 | 0.85 | 0.57 |
| IM | 0.87 | 0.81 | 0.88 | 0.90 | 0.95 | 0.86 |
| DYS | 0.41 | 0.18 | 0 | 0 | 0.49 | 0 |
| - LGD | 0.18 | 0.20 | 0 | 0 | 0.27 | 0 |
| - HGD | 0.55 | 0 | . § | . § | 0 | . § |

* Targeted and random biopsies; # Random biopsies; § No patients were diagnosed with HGD in antrum, angulus or cardia; AG: atrophic gastritis; IM: intestinal metaplasia; DYS: dysplasia; LGD: low grade dysplasia; HGD: high grade dysplasia;

**Table 5.** Interobserver agreement (kappa values) for different stages of the OLGA and OLGIM staging system

| | OLGA | OLGIM |
|---|---|---|
| Stages: | | |
| 0-IV | 0.38 | 0.58 |
| 0 | 0.56 | 0.88 |
| I | 0.19 | 0.48 |
| II | 0.29 | 0.31 |
| III | 0.36 | 0.48 |
| IV | 0.48 | 0.59 |
| III-IV | 0.48 | 0.61 |

### *OLGA versus OLGIM*

Eighty-four patients (67%) were classified as stage I-IV according to the OLGA (stage I, n = 24; stage II, n = 30; stage III, n = 22; stage IV, n = 8) and 79 patients (63%) were classified as stage I-IV according to the OLGIM (stage I, n = 22; stage II, n = 28; stage III, n = 20; stage IV, n = 9) (*P* = .23). The baseline characteristics were not significantly different between the stages 0-IV of the OLGA and the stages 0-IV of the OLGIM (Table 3). In total, 30 patients (24%) clustered in stage III-IV in the OLGA and 29 patients (23%) clustered in stage III-IV in the OLGIM.

Table 6 demonstrates the differences between patient distribution in the stages 0-IV according to the OLGA and patient distribution in the stages 0-IV according to the OLGIM. Overall, in 104 patients (83%) the gastric cancer risk was classified equally in the OLGIM and the OLGA. The gastric cancer risk of 13 patients (10%) was downgraded with the OLGIM compared with the OLGA, whereas 8 patients (6%) were classified as having a higher risk (Table 6). Among the 13 patients that were downgraded according to the OLGIM, the most severe grade of IM was mild in 3 patients (23%), moderate in 1 patient (8%), and marked in 1 patient (8%). The remaining 8 patients (62%) demonstrated no IM, but marked AG in 1 patient (8%), moderate AG in 1 patient (8%), and mild AG in 6 patients (46%). Within the group of 8 patients who were classified as having a higher gastric cancer risk according to the OLGIM, the most severe grade of IM was mild in 3 patients (37.5%) and moderate in 2 patients (25%). In addition, 3 patients (37.5%) had a most-severe diagnosis of marked IM, of which 2 also had a diagnosis of low-grade dysplasia.

**Table 6.** Patient distribution in the OLGA staging system *versus* the OLGIM staging system

| | | OLGIM | | | | | Total |
|---|---|---|---|---|---|---|---|
| | | Stage 0 | Stage 1 | Stage 2 | Stage 3 | Stage 4 | |
| OLGA | Stage 0 | 38 | 3 | 0 | 0 | 0 | 41 |
| | Stage 1 | 6 | 16 | 1 | 1 | 0 | 24 |
| | Stage 2 | 1 | 2 | 25 | 2 | 0 | 30 |
| | Stage 3 | 1 | 1 | 2 | 17 | 1 | 22 |
| | Stage 4 | 0 | 0 | 0 | 0 | 8 | 8 |
| Total | | 46 | 22 | 28 | 20 | 9 | 125 |

Of the dysplasia patients, 5 patients (4%) had a diagnosis of low-grade dysplasia and 2 patients (2%) had a diagnosis of high-grade dysplasia. The prevalence of dysplasia in stage III-IV was 17% (5/30) and 21% (6/29) for the OLGA and the OLGIM, respectively (Table 3). Both patients with high-grade dysplasia clustered in stage III-IV of the OLGA as well as the OLGIM. Out of the low-grade dysplasia patients, one patient was reclassified in stage III according to the OLGIM instead of stage I according to the OLGA, and 1 patient was reclassified in stage IV according to the OLGIM instead of stage III in the OLGA. A significant association was demonstrated between the severity of gastritis staging based on dysplasia and the stages I-IV in the OLGA as well as between the severity of gastritis based on dysplasia grading and the stages I-IV in the OLGIM ($P = .02$ and $P = .001$, respectively). In addition, considering together stages 0-II versus stages III-IV also resulted in a significant association between stages III-IV and dysplasia for the OLGA as well as the OLGIM ($P < .01$ and $P < .001$, respectively).

In the analysis of patients with gastric cancer, 10 patients (50%) were diagnosed with intestinal-type gastric cancer and 10 patients (50%) with diffuse-type gastric cancer. Of the 10 intestinal-type gastric cancer patients, 5 (50%) were classified in stage III-IV of both the OLGA and OLGIM, and 5 (50%) were classified in stage 0-II of both OLGA and OLGIM. Out of the 10 diffuse type gastric cancer patients, 1 (10%) was classified in stage III-IV of both OLGA and OLGIM, whereas 9 (45%) were classified in stage 0-II of both OLGA and OLGIM. In addition, significantly more patients with intestinal-type gastric cancer were classified in stage III-IV of OLGA and OLGIM than those with diffuse-type gastric cancer patients ($P = .05$).

## DISCUSSION

This study demonstrates that replacement of AG by IM in the staging of gastritis increases interobserver agreement considerably. In addition, the correlation with the severity of gastritis remains at least as strong. Therefore, the OLGIM may be preferred over the OLGA for the prediction of gastric cancer risk in patients with pre-malignant gastric lesions.

Endoscopic follow-up of premalignant gastric lesions should be limited to patients at high cancer risk. *H. pylori* virulence, environmental factors, and the presence of concomitant associated lesions are well-known risk factors.[19-23] In addition, the intragastric extent, distribution, and severity of premalignant gastric lesions have consistently been related to gastric cancer risk. For instance, the severity and extent of IM are important predictors of gastric cancer risk,[16,24-27] with a more than 5-fold increased risk of gastric adenocarcinoma in patients with IM involving the lesser curvature of the corpus.[25] However, diagnoses of AG, IM, and even dysplasia are often disregarded in clinical practice.[3] Recently, the OLGA system was proposed to improve clinical relevance of histologic findings regarding prognosis, therapy, and management of patients with premalignant gastric lesions.[6] Although this system has

great potential in guiding clinical decisions, the use of AG as the principal parameter may be its major drawback, most importantly for reasons of reproducibility.

The present study shows that the level of agreement on a diagnosis of AG according to the Sydney classification is moderate at best. In contrast, agreement on the presence of IM was almost perfect. These observations are in line with earlier studies. Despite the simple definition of atrophy and the introduction of visual analog scales, the agreement for presence and grading of AG was slight to moderate (kappas for AG ranged from 0.08 to 0.5), whereas agreement on the diagnosis of IM was substantial to almost perfect (kappa values from 0.68 to 0.92).[10,11,14,28] As was shown in earlier studies, we demonstrated that improved agreement was observed for AG from the oxyntic mucosa biopsies compared with biopsies from the antrum, which is explained by the small number of gastric glands in normal antral mucosa.[12,29,30]

Gastric dysplasia is often a difficult histologic diagnosis, which results in poor interobserver agreement.[31] In addition, geographic differences exist for the assessment of dysplasia and gastric cancer between the East and the West, despite the introduction of classification systems.[5,13, 32-34] The present study confirmed that agreement for low-grade dysplasia still remains extremely poor (kappa value 0.2), whereas for high-grade dysplasia, agreement was moderate (kappa value 0.6). The disagreement for the diagnosis of low-grade dysplasia among our 3 expert pathologists implies that clinical decisions in difficult cases may not benefit from multiple expert opinions. In contrast, a third expert opinion adds to agreement on the diagnosis of high-grade dysplasia and may guide clinical decisions on surveillance or intervention.

In this study, IM was proposed as marker for assessing gastric cancer risk. IM is the next step in the Correa model for gastric cancer development.[1] In this model, AG progresses to IM, which can progress to dysplasia and eventually to gastric adenocarcinoma over a time frame of several years to decades. The effectiveness of IM instead of AG in predicting gastric cancer remains dependent on the reproducibility for this proposed marker and the inclusion of a subpopulation of patients at high gastric cancer risk. The present study shows that in line with the higher interobserver agreement for IM compared with AG, the replacement of AG in the OLGA by IM (OLGIM) improves reproducibility and thus leads to a more consistent gastric cancer risk assessment in patients with premalignant gastric lesions. With this adaptation, fewer patients were categorized in stage I-IV and particularly in stages III-IV in the OLGIM, creating a smaller population for whom surveillance should be considered. In addition, the correlation between the severity of gastritis for OLGA and OLGIM stages remains at least as strong for AG, IM, and dysplasia patients. For these reasons, the OLGIM may result in a smaller and better-defined subpopulation of patients at risk of gastric cancer compared with the OLGA. As a result, use of the OLGIM might lead to more feasible and cost-effective surveillance strategies for patients at risk of gastric cancer and a more consistent gastric cancer risk assessment. This is clinically relevant, because gastric cancer remains a common condition,

but other than with conditions like Barrett's esophagus or colonic adenomas, most endoscopists do not know how to manage patients with premalignant gastric lesions.

Emphasizing the OLGIM as an additional parameter to the OLGA rather than an alternative parameter seems unjustified. However, in clinical decision making, the histologic system should preferably be combined with individual risk factors for gastric cancer,[16,35,36] as was illustrated by 2 cases with moderate and marked AG, which were downgraded according to the OLGIM compared with staging with the OLGA.

A few limitations of our study warrant consideration. First, although both OLGA and OLGIM stages 0-II were common in the mucosa surrounding gastric cancer, we did not demonstrate significant differences between both classifications; however, we included only a small number of patients. Therefore, this analysis supports our main finding that assessment of AG may be replaced by assessment of IM when staging gastritis. Large prospective studies with adequate follow-up, in several countries with a wide spectrum of gastric cancer incidences, are necessary to confirm our data and to evaluate the prognostic value of both staging systems.[8,9] Second, 3 expert GI pathologists assessed gastric biopsies in this study. Therefore, interobserver agreement may be higher than in routine clinical practice. However, the far-from-perfect kappa values for AG emphasize that gastritis staging according to the OLGA should probably not be introduced for routine assessment. Third, we obtained 12 biopsy specimens instead of 5 biopsy specimens according to the Sydney classification. However, it remains controversial whether those 5 biopsy specimens are sufficient for an adequate diagnosis of IM and dysplasia.[4,37] Moreover, owing to the sometimes patchy distribution of premalignant lesions, the risk of missing these lesions is high. Therefore, we think that our biopsy strategy in the present study is justified, and that correlation between OLGIM stages and gastric cancer risk increases with this strategy. Finally, it remains unclear which patients with dysplasia will develop gastric cancer. However, an earlier large study demonstrated that 4% to 33% of patients with mild to severe dysplasia develop gastric cancer within 10 years.[3] Because the OLGIM seems to predict dysplasia more adequately than the OLGA, the use of this new staging system may lead to optimal patient identification with the aim of further reducing gastric cancer incidence in the future.

In conclusion, IM staging yields more accurate results regarding reproducibility and at least as strong results in assessing the severity of the disease compared with AG staging. These observations support the use of the proposed OLGIM for gastric cancer risk assessment instead of the OLGA, and provide clinicians with an easy tool to identify patients with advanced premalignant gastric lesions. However, owing to the lack of long-term outcomes and the relatively small number of patients with gastric cancer included in this study, larger long-term prospective studies are needed to confirm the correlation between OLGIM stages and gastric cancer risk.

## ACKNOWLEDGEMENTS

The permission of the American Journal of Surgical Pathology for reusing the visual analog scale of the updated Sydney classification is kindly acknowledged.

## REFERENCES


1 Correa P. Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 1992;52:6735-40.

2 Kuipers EJ. Review article: exploring the link between Helicobacter pylori and gastric cancer. *Aliment Pharmacol Ther* 1999;13 Suppl 1:3-11.

3 de Vries AC, van Grieken NC, Looman CW, *et al.* Gastric cancer risk in patients with premalignant gastric lesions: a nationwide cohort study in the Netherlands. *Gastroenterology* 2008;134:945-52.

4 Dixon MF, Genta RM, Yardley JH, *et al.* Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996;20:1161-81.

5 Dixon MF. Gastrointestinal epithelial neoplasia: Vienna revisited. *Gut* 2002;51:130-1.

6 Rugge M, Genta RM. Staging gastritis: an international proposal. *Gastroenterology* 2005;129:1807-8.

7 Rugge M, Correa P, Di Mario F, *et al.* OLGA staging for gastritis: a tutorial. *Dig Liver Dis* 2008;40: 650-8.

8 Rugge M, Meggio A, Pennelli G, *et al.* Gastritis staging in clinical practice: the OLGA staging system. *Gut* 2007;56:631-6.

9 Satoh K, Osawa H, Yoshizawa M, *et al.* Assessment of atrophic gastritis using the OLGA system. *Helicobacter* 2008;13:225-9.

10 el-Zimaity HM, Graham DY, al-Assi MT, *et al.* Interobserver variation in the histopathological assessment of Helicobacter pylori gastritis. *Hum Pathol* 1996;27:35-41.

11 Chen XY, van der Hulst RW, Bruno MJ, *et al.* Interobserver variation in the histopathological scoring of Helicobacter pylori related gastritis. *J Clin Pathol* 1999;52:612-5.

12 Offerhaus GJ, Price AB, Haot J, *et al.* Observer agreement on the grading of gastric atrophy. *Histopathology* 1999;34:320-5.

13 de Vries AC, Haringsma J, Kuipers EJ. The detection, surveillance and treatment of premalignant gastric lesions related to Helicobacter pylori infection. *Helicobacter* 2007;12:1-15.

14 Guarner J, Herrera-Goepfert R, Mohar A, *et al.* Interobserver variability in application of the revised Sydney classification for gastritis. *Hum Pathol* 1999;30:1431-4.

15 Cote RA, Robboy S. Progress in medical information management. Systematized nomenclature of medicine (SNOMED). *Jama* 1980;243:756-62.

16 de Vries AC, Haringsma J, de Vries RA, *et al.* The use of clinical, histological and serological parameters to predict the intragastric extent of intestinal metaplasia: a recommendation for routine practice. *Gastointestinal Endoscopy* 2008;in press.

17 Fleiss JL. Statistical Methods for Rates and Proportions (ed 2). *New York, NY, Wiley* 1981.

18 Landis RJ KG. The measurements of observer agreement for categorical data. . *Biometrics* 1977;33: 159-74.

19 Kuipers EJ, Perez-Perez GI, Meuwissen SG, *et al.* Helicobacter pylori and atrophic gastritis: importance of the cagA status. *J Natl Cancer Inst* 1995;87:1777-80.

20 Kato I, Vivas J, Plummer M, *et al.* Environmental factors in Helicobacter pylori-related gastric precancerous lesions in Venezuela. *Cancer Epidemiol Biomarkers Prev* 2004;13:468-76.

21 Capelle LG, de Vries AC, Looman CW, *et al.* Gastric MALT lymphoma: epidemiology and high adenocarcinoma risk in a nation-wide study. *Eur J Cancer* 2008;44:2470-6.

22 Lamarque D, Levy M, Chaumette MT, *et al.* Frequent and rapid progression of atrophy and intestinal metaplasia in gastric mucosa of patients with MALT lymphoma. *Am J Gastroenterol* 2006;101: 1886-93.

23 Hansson LE, Nyren O, Hsing AW, *et al.* The risk of stomach cancer in patients with gastric or duodenal ulcer disease. *N Engl J Med* 1996;335:242-9.

24 Leung WK, Lin SR, Ching JY, *et al.* Factors predicting progression of gastric intestinal metaplasia: results of a randomised trial on Helicobacter pylori eradication. *Gut* 2004;53:1244-9.

25 Cassaro M, Rugge M, Gutierrez O, *et al.* Topographic patterns of intestinal metaplasia and gastric cancer. *Am J Gastroenterol* 2000;95:1431-8.

26 El-Zimaity HM, Ramchatesingh J, Saeed MA, *et al.* Gastric intestinal metaplasia: subtypes and natural history. *J Clin Pathol* 2001;54:679-83.

27 Filipe MI, Munoz N, Matko I, *et al.* Intestinal metaplasia types and the risk of gastric cancer: a cohort study in Slovenia. *Int J Cancer* 1994;57:324-9.

28 Correa P. Chronic gastritis: a clinico-pathological classification. *Am J Gastroenterol* 1988;83:504-9.

29 van Grieken NC, Weiss MM, Meijer GA, *et al.* Rapid quantitative assessment of gastric corpus atrophy in tissue sections. *J Clin Pathol* 2001;54:63-9.

30 van Grieken NC, Meijer GA, Kale I, *et al.* Quantitative assessment of gastric antrum atrophy shows restitution to normal histology after Helicobacter pylori eradication. *Digestion* 2004;69:27-33.

31 Sarela AI, Scott N, Verbeke CS, *et al.* Diagnostic variation and outcome for high-grade gastric epithelial dysplasia. *Arch Surg* 2005;140:644-9.

32 Schlemper RJ, Itabashi M, Kato Y, *et al.* Differences in diagnostic criteria for gastric carcinoma between Japanese and western pathologists. *Lancet* 1997;349:1725-9.

33 Rugge M, Correa P, Dixon MF, *et al.* Gastric dysplasia: the Padova international classification. *Am J Surg Pathol* 2000;24:167-76.

34 Schlemper RJ, Riddell RH, Kato Y, *et al.* The Vienna classification of gastrointestinal epithelial neoplasia. *Gut* 2000;47:251-5.

35 Watabe H, Mitsushima T, Yamaji Y, *et al.* Predicting the development of gastric cancer from combining Helicobacter pylori antibodies and serum pepsinogen status: a prospective endoscopic cohort study. *Gut* 2005;54:764-8.

36 El-Omar EM, Oien K, Murray LS, *et al.* Increased prevalence of precancerous changes in relatives of gastric cancer patients: critical role of H. pylori. *Gastroenterology* 2000;118:22-30.

37 El-Zimaity HM, Graham DY. Evaluation of gastric mucosal biopsy site and number for identification of Helicobacter pylori or intestinal metaplasia: role of the Sydney System. *Hum Pathol* 1999; 30:72-7.

# Chapter 8

## Risk and epidemiological time trends of gastric cancer in Lynch syndrome carriers in the Netherlands

Lisette G. Capelle[1], Nicole C. T. Van Grieken[2], Hester F. Lingsma[3], Ewout W. Steyerberg[3], Willem J. Klokman[4], Marco J. Bruno[1], Hans F. A. Vasen[5,6], Ernst J. Kuipers[1,7]

Departments of [1]Gastroenterology and Hepatology, [3]Public Health, and [7]Internal Medicine, Erasmus MC University Medical Center, Rotterdam, [2]Department of Pathology, VU University Medical Center, Amsterdam, [4]Department of Epidemiology, The Netherlands Cancer Institute, Amsterdam, [5]The Netherlands Foundation for the Detection of Hereditary Tumors, [6]Department of Gastroenterology, Leiden University Medical Center, Leiden, the Netherlands.

*Gastroenterology; 2010;138(2):487-92*

## ABSTRACT

*Background & Aims:* Although gastric cancer forms part of the Lynch syndrome tumor spectrum, the risk of developing gastric cancer in Lynch syndrome families is unknown, resulting in a lack of clear guidelines for surveillance. The aim of this study was to evaluate incidence trends and risk of developing gastric cancer among Lynch syndrome mutation carriers in a Western population.

*Methods:* Lynch syndrome mutation carriers were selected from the Dutch Hereditary Cancer Registry. The gastric cancer incidence in Lynch syndrome mutation carriers was compared to the gastric cancer incidence in the Dutch population between 1970 and 2003. Standardized incidence ratios were calculated by a Poisson model. Cumulative risks were calculated by Kaplan-Meier analysis.

*Results:* Overall, 2014 Lynch syndrome mutation carriers were identified. Gastric cancer was diagnosed in 32 (1.6%) subjects (male/female: 21/11), 22 (69%) of them had a negative family history of gastric cancer. The standardized incidence ratios of gastric cancer was 3.4 (95% confidence interval, 2.1–5.2) and showed a nonsignificant decline between 1970 and 2003 ($P = .30$). Absolute risk of developing gastric cancer also showed no significant change over time ($P = .51$). Lifetime risk of developing gastric cancer was 8.0% in males vs 5.3% in females ($P = .02$), and 4.8% and 9% for MLH1 and MSH2 carriers, respectively. None of the 378 MSH6 carriers developed gastric cancer ($P = .002$ vs MLH1 and MSH2 combined lifetime risk).

*Conclusions:* Lynch syndrome mutation carriers have a substantial risk for gastric cancer, in particular patients with an MLH1 or MSH2 mutation. Family history for gastric cancer is a poor indicator for individual risk. Surveillance gastroscopy for Lynch syndrome patients carrying an MLH1 or MSH2 mutation should therefore be considered.

## INTRODUCTION

Hereditary nonpolyposis colorectal cancer (also known as Lynch syndrome) is the most common dominantly inherited colorectal cancer syndrome.[1] This syndrome is caused by germ-line mutations in 4 mismatch repair genes (MLH1, MSH2, MSH6, and PMS2), resulting in development of a spectrum of different tumors.[2-4] These in particular include colorectal cancer and endometrial cancer, with a lifetime risk of 60–80% and 30–70%, respectively.[5] Consequently, screening of Lynch syndrome families for these types of cancer has been widely accepted.

Apart from these commonly associated cancers, gastric cancer also forms part of the Lynch syndrome tumor spectrum.[6-10] However, incidence of Lynch syndrome–associated gastric cancer seems to depend on geography and time. A century ago, with the first description of Lynch syndrome, gastric cancer did predominate over colorectal cancer, with the syndrome presenting as hereditary gastric cancer.[11] Today, in Western countries, gastric cancer is, among Lynch syndrome carriers, far less common than colorectal cancer and occurs, on average, at an older age.

However, because clear incidence rates are lacking and the actual risk of gastric cancer in Lynch syndrome families is largely unknown, surveillance strategies for gastric cancer in Lynch syndrome are controversial. Therefore, the aim of this study was to determine whether surveillance of gastric cancer is indicated in Lynch syndrome families in a Western population by evaluating incidence trends and relative and cumulative gastric cancer risks for known and putative Lynch syndrome mutation carriers.

## METHODS

### *Hereditary Cancer Registry*

In the Netherlands, families with Lynch syndrome are registered at the nationwide Dutch Hereditary Cancer registry. A detailed description of the registry's approach has been described elsewhere.[12] Briefly, families suspected for Lynch syndrome are referred by specialists and genetic centers to the registry from all parts of the Netherlands. In the 1980s, genetic field workers worked in cooperation with clinical and genetic centers to perform genealogical studies. Today, analysis of the families is performed by the collaborating cancer family clinics at university centers in the Netherlands. The main goal of the registry is to promote early detection of cancer in high-risk families. Therefore, all individual family members are offered registration after confirmation of diagnosis of Lynch syndrome. Written informed consent is required for registration. The registry not only serves as information and counseling service, but also registers adherence to and outcomes of surveillance of all registered patients.

## *Patient Selection and Data Collection*

Registered families were eligible for the study if at least 1 family member was identified with a germ-line mutation in one of the mismatch repair genes. Of 236 families with an identified mutation, 81 families harbored an MLH1 mutation, 105 an MSH2 mutation, 49 an MSH6 mutation, and 1 family harbored a PMS2 mutation. All proven mutation carriers and putative carriers were selected. If subjects tested positive for a mismatch repair gene mutation or when subjects were obligatory to be a mutation carrier because of their position in the pedigree, they were considered to be mutation carriers. Putative carriers were family members who had not undergone genetic testing but had been diagnosed with colorectal or endometrial cancer before the age of 60 years.

For all subjects, data were collected on the following variables: date of birth, date of gastric cancer diagnosis, date of death, gender, type of mutation in family, mutation status, and family history of gastric cancer. Information of dates of birth was lacking in 7% of cases. Unknown birth dates were imputed based on the known birth dates in each generation of the family and based on pedigree structure. For horizontal imputing, the birth date in each generation was estimated based on the known birth dates and this estimated birth date was then applied to siblings with unknown birth dates. For vertical imputing, the average age difference between generations within each birth cohort was calculated. This estimated age difference between generations was used to impute birth dates of children or parents in case their birth dates and those of each of their siblings were unknown. Subjects of families with >80% missing birth dates or subjects with unknown gender were excluded. Furthermore, because critical data (eg, date of birth, date of death, gender) were less reliable and often missed for subjects born before January 1, 1900, these subjects were excluded. Verification (pathology report, medical report, or family report) was collected for each gastric cancer diagnosis.

## *Statistical Analysis*

### *Standardized Incidence Ratios*

As reliable population-specific incidence rates of gastric cancer in the Netherlands were available from January 1, 1970 onward, the standardized incidence ratios (SIRs) of gastric cancer were only calculated for patients who were alive at or after January 1, 1970. In addition, patients with a gastric cancer diagnosis or date of death or date of last contact before January 1, 1970, were excluded from this analysis. Time at risk started at January 1, 1970 and ended at date of gastric cancer diagnosis, date of death, date of last contact, or January 1, 2003. Incidence of gastric cancer observed in known and putative mutation carriers was compared to the incidence of gastric cancer in the general Dutch population from 1970 until 2003 and aggregated over age and gender. In this person-years type of analysis, the ratio of observed and expected number of gastric cancers in the study cohort was evaluated. Ninety-

five percent confidence intervals (CI) were calculated assuming that the number of observed cases followed a Poisson distribution. The Poisson Trend statistic was calculated to evaluate time trends of gastric cancer in Lynch syndrome mutation carriers.[13]

*Risk Assessment*

Cumulative risks of developing gastric cancer were estimated as a function of time using the Kaplan-Meier method. For this analysis, all subjects of the cohort were included. Subjects were studied with respect to their risk of developing gastric cancer from birth to death. Observation time ended at gastric cancer diagnosis, date of death, date of last contact or the closing date of the study, ie, January 1, 2008. Differences in survival curves were tested for statistical significance by the log-rank test, for male vs female and mutation status. A Cox regression model including a time-dependent covariate was used to assess the effect of calendar-year on gastric cancer risk.

## RESULTS

In total, 2014 (male/female: 948/1066) mutation carriers of 236 families were identified, including 1511 known and 503 putative mutation carriers. During follow-up, 32 (1.6%) (known and putative) mutation carriers were diagnosed with gastric cancer at a median age of 55 years (range, 27–82 years), 87.5% of them developed gastric cancer at 45 years of age or older (Table 1).

**Table 1.** Baseline characteristics of 236 Lynch families

| Characteristic | Total (n) | % | Gastric cancer (n) |
|---|---|---|---|
| All | 2014 | | 32 |
| Sex | | | |
| - Male | 948 | 47 | 21 |
| - Female | 1066 | 53 | 11 |
| Mutation status | | | |
| - Carrier | 1511 | 75 | 25 |
| - Putative carrier | 503 | 25 | 7 |
| Mutation in family | | | |
| - MLH1 | 737 | 37 | 12 |
| - MSH2 | 897 | 44 | 20 |
| - MSH6 | 378 | 19 | -- |
| - PMS2 | 2 | 0.1 | -- |

In 22 families (9.3%) only 1 patient was diagnosed with gastric cancer, in 3 families (1.2%) 2 patients were diagnosed with gastric cancer, and in 1 family (0.4%) 4 patients were diagnosed with gastric cancer. Twenty-five (1.6%) of 1511 known mutation carriers were diagnosed with gastric cancer and 7 (1.4%) of 503 putative mutation carriers. Mean age of gastric cancer

development was not significantly different for putative and known mutation carriers (mean age, 53.7 years vs 54.6 years, respectively; *P* = .87). Of 32 subjects with gastric cancer, 12 gastric cancers occurred among 737 MLH1 mutation carriers and 20 among 897 MSH2 mutation carriers (*P* = .38). No gastric cancer was diagnosed among 378 MSH6 mutation carriers (*P* = .006 value in comparison with combined MLH1 and MSH2).

In 14 (43%) patients, gastric cancer was the first and only malignancy. Mean survival of these patients was short (approximately 10 months [standard deviation, 2.1 years]). In 18 (56%) subjects, other cancers occurred metachronously during a mean survival after the diagnosis of gastric cancer of 3.9 years (standard deviation, 7.6 years). In 7 of these 18 subjects, gastric cancer was the first malignancy. Other first or second malignancies were colorectal cancer (n = 15), endometrial cancer (n = 2), and skin cancer (n = 1). Third malignancies occurred in 8 (25%) subjects, which included colorectal cancer (n = 3), breast cancer (n = 1), prostate cancer (n = 1), brain cancer (n = 1), bladder cancer (n = 1), and esophageal cancer (n = 1).

### *Histology*

Gastric cancer diagnosis was based on histological reports in 22 (69%) subjects, on medical reports in 4 (12%) subjects, and on family reports in the remaining 6 (19%) subjects. Of the 26 patients with gastric cancer confirmed by histological or medical reports, 4 (15%) demonstrated gastric cancer in the antrum, 9 (35%) in the corpus, and 6 (23%) were located in the cardia, in the remaining 7 (27%) patients' gastric cancer location was not described. According to the Lauren classification, 16 (62%) of the patients with confirmed gastric cancer suffered from an intestinal type gastric cancer and 6 (23%) from a diffuse-type gastric cancer. From the remaining 4 (15%) patients, the histology or medical report did not specify the histological subtype.[14] The tumor was already invading the lamina propria, the muscularis propria, or beyond in 19 (73%) mutation carriers, and lymph nodes were involved in 7 (27%) of these patients.

### *Relative Risk of Gastric Cancer*

Overall, SIR was 3.4 (95% CI, 2.1–5.2), with a somewhat higher SIR for males (3.8; 95% CI, 2.1–6.3) than for females (2.7; 95% CI, 0.98–5.8) (Table 2). Gastric cancer risk was 2.9 (95% CI, 1.1–6.3) times higher in mutation carriers with an identified MLH1 mutation, whereas in mutation carriers with an identified MSH2 mutation the relative risk of developing gastric cancer was 6.1 times (95% CI, 3.3–10.2) increased in comparison with the general population. Of the gastric cancer subjects, 20 (63%) developed gastric cancer between 1970 and 2003; these subjects formed the basis for the SIR calculations. The SIR showed a declining pattern from 4.0 (95% CI, 1.5–8.6) in the years 1970–1979, to 3.0 (95% CI, 1.1–6.6) in 1980–1989, to 2.1 (95% CI, 0.6–5.3) in 1990–1999; however, this trend was not statistically significant (*P* = .30).

**Table 2.** Standardized incidence ratios (SIRs) between 1970 and 2003 of developing gastric cancer in Lynch Syndrome families compared to the Dutch population

| Characteristic | Observed cases | SIR | 95% CI |
|---|---|---|---|
| All | 20 | 3.4 | 2.1-5.2 |
| Sex | | | |
| - Male | 14 | 3.8 | 2.1-6.3 |
| - Female | 6 | 2.7 | 0.98-5.8 |
| Mutation status | | | |
| - Carrier | 16 | 3.9 | 2.2-6.4 |
| - Putative carrier | 4 | 2.1 | 0.6-5.4 |
| Mutation in family | | | |
| - MLH1 | 6 | 2.9 | 1.1-6.3 |
| - MSH2 | 14 | 6.1 | 3.3-10.2 |

### *Cumulative Gastric Cancer Risk*

In the total cohort, the absolute risk of developing gastric cancer for Lynch syndrome patients remained nearly constant during the previous decades, with a statistically nonsignificant decrease with an odds ratio of 0.923 per decade (95% CI, 0.82–1.10) ($P = .51$). A Kaplan-Meier analysis demonstrated a lifetime risk of developing gastric cancer of 8.0% for males, and 5.3% for females ($P = .02$) (Table 3 and Figure 1). Furthermore, Figure 2 shows that no patients with MSH6 mutations developed gastric cancer, which contrasted with the increased lifetime risk in both patients with an MLH1 and those with an MSH2 mutation ($P = .002$). However, the risk did not differ between MLH1 and MSH2 mutation carriers ($P = .26$).

**Table 3.** Cumulative incidence of gastric cancer development per 10-years age groups and gender in Lynch syndrome mutation carriers

| | Cumulative incidence (%) | |
|---|---|---|
| | **Male [95% CI]** | **Female (95% CI)** |
| **Age groups** | | |
| ≤ 40 yrs | 0.2 [NA-0.6] | 0.1 [NA-0.3] |
| 50 yrs | 1.5 [0.5-2.5] | 0.4 [-0.1-0.9] |
| 60 yrs | 3.0 [1.4-4.6] | 2.0 [0.6-3.3] |
| 70 yrs | 6.2 [3.2-9.2] | 2.0 [0.6-3.3] |
| 80 yrs | 8.0 [3.4-12.5] | 2.8 [0.7-4.9] |
| **Lifetime risk** | 8.0 [3.4-12.5] | 5.3* [NA-10.7] |

Legend: NA; not applicable, *One female mutation carrier developed gastric cancer >80 years.

## DISCUSSION

In order to develop and target optimal prevention strategies for gastric cancer in Lynch syndrome families, accurate data concerning incidence trends, as well as relative and age-specific risks are essential. This study provides such data and first shows that Lynch syndrome subjects are indeed at an increased risk of developing gastric cancer. Secondly, because of

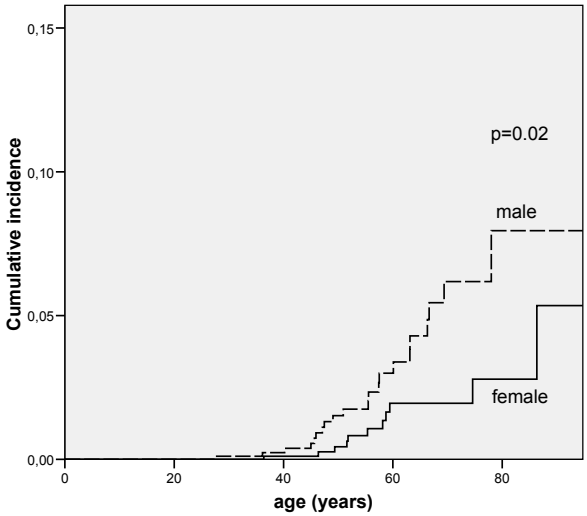


**Figure 1.** Cumulative incidence of gastric cancer in male and female Lynch syndrome carriers

the nonsignificant decreasing pattern of SIRs, we found no convincing evidence of a declining trend of gastric cancer incidence. Thirdly, patients with an MLH1 or MSH2 mutation have a substantial cumulative risk of developing gastric cancer during their lifetime, whereas no gastric cancers were observed among MSH6 mutation carriers in this nationwide long-term observational study. Finally, the majority of subjects with gastric cancer had a negative family history of gastric cancer.

The first description of a family with the "family cancer syndrome," now known as Lynch syndrome, was published in 1913.[11] In this family, designated as "Family G," gastric cancer was the predominant lesion. However, subsequent generations of this family demonstrated a declining incidence of gastric cancer (paralleling its declining prevalence in the general population) and an increasing incidence of colorectal cancer.[11,15,16]

In the Netherlands, the incidence of gastric cancer is rapidly declining from 24.0 per 100.000 people in 1989 to 13.5 per 100.000 people in 2006 (European standardized rates).[17] This decline is primarily attributed to the declining *Helicobacter pylori* prevalence in Western countries. It may be that observed changes over time in gastric cancer incidence in specific Lynch syndrome families also relate to the changing epidemiology of *H. pylori* and other environmental risk factors. The tumor spectrum among Lynch syndrome patients would then, to some extent, reflect the cancer incidence in the general population. This is in line with a Korean study that demonstrated a very high relative risk of gastric cancer for Lynch syndrome subjects in a country with a high *H. pylori* prevalence.[18,19] The nonsignificant declining pattern of SIRs in our Western Lynch syndrome population, however, shows that in recent decades the incidence of gastric cancer is not decreasing further.

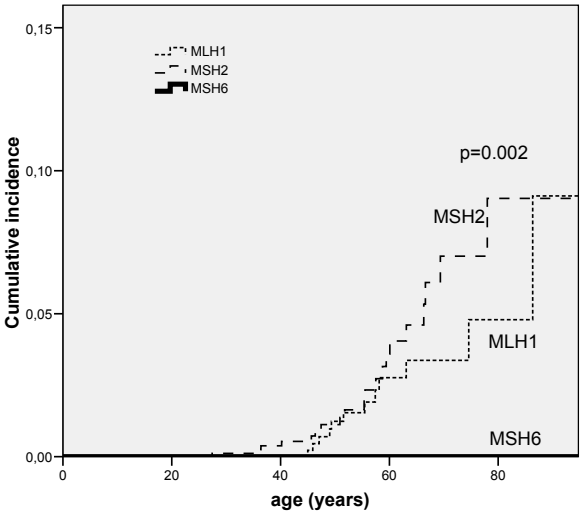


**Figure 2.** Cumulative incidence of gastric cancer for MLH1, MSH2 and MSH6 mutation carriers

In our study, gender and mutation status were identified as risk factors for gastric cancer in Lynch syndrome mutation carriers. Gender differences were not markedly related to a higher gastric cancer risk in previous studies performed in Western Countries.[20,21] An explanation for the high male risk found in our study is the fact that males in the general Dutch population are already at a higher risk of developing gastric cancer. Less use of nonsteroidal anti-inflammatory drugs and more smoking in absolute numbers in male patients are clarifications for this male predominance. These factors seem to influence gastric cancer risk both in the general population as well as in the Lynch syndrome cohort. Hence, gender differences found in this study partly reflect the higher lifetime risk of developing gastric cancer in the general population.[17]

Consistent with previous studies, MSH2 mutation carriers tended to have a higher risk of developing gastric cancer than MLH1 carriers.[21-25] In contrast to both MLH1 and MSH2 mutation carriers, our findings suggest that MSH6 mutation carriers seem to have a low gastric cancer risk. Characteristics of MSH6 mutation carriers include a high incidence of endometrial cancer, which also occurs at an older age as compared to MLH1 and MSH2 carriers. Moreover, no small bowel tumors were observed in these patients.[23,25-28] Similar to these latter observations, no patients with an MSH6 mutation developed gastric cancer in our study. Because of the small number of MSH6 carriers included in our study and previous studies, additional investigation is necessary to confirm the low risk of MSH6 mutations.

Although clear evidence is lacking, previous studies recommended targeting upper gastrointestinal surveillance endoscopy for Lynch syndrome, particularly in those families with more than 1 family member with gastric cancer.[4,5,29,30] Our study shows that the majority of gastric cancers occurred as single cases within Lynch syndrome families. First, these observa-

tions indicate that family history is a poor determinant to offer or withhold surveillance for gastric cancer in Lynch syndrome mutation carriers. Second, these observations point out that SIR estimates in this study are not affected by inherent correlation of risk factors within families, such as behavioral or environmental risk factors. This is further supported by a previous study that was based on a cohort of 6041 high-risk members of families with known MLH1 and MSH2 mutations from Western countries, demonstrating no clustering for gastric cancer diagnosis among families.[7,21]

A few limitations of our study warrant consideration. First, results concerning gastric cancer risk are probably slightly underestimated because we included only confirmed and putative mutation carriers. This means that we likely excluded certain cases, in particular from the first half of the century.[23] Second, gastric cancer diagnoses were not confirmed by a pathology or medical report in 6 patients. However, because previous studies demonstrated a high reliability for a family history of cancer for first-degree relatives, and moderate reliability for second- or third-degree relatives, the addition of the gastric cancers confirmed by family history seems justified.[31] In addition, the inclusion of phenocopies or sporadic cases cannot be excluded in this study, in particular, not for the putative mutation carriers. However, because mean age was not significantly different for these patients, the inclusion of these patients seems justified. Third, from the 22 patients for whom data concerning histology was available, 6 (27%) demonstrated diffuse-type gastric cancer. This finding is striking because in previous studies, diffuse-type gastric cancer in Lynch syndrome was rather exceptional and surveillance recommendations are particularly useful for patients with intestinal-type gastric cancer because of the detection of premalignant lesions.[8,32]

Surveillance of Lynch syndrome mutation carriers may lead to a further decline in gastric cancer incidence. However, whether surveillance should be provided to Lynch syndrome mutation carriers remains controversial. Because of the high gastric cancer risk in younger age groups and the high incidence in the general population, gastric cancer screening is recommended for Lynch syndrome carriers in Korea and Japan.[18] In Western countries the situation varies. A Finnish study reported a high incidence of intestinal-type gastric cancer in Lynch syndrome families, but screening was not recommended because of poor cost-effectiveness.[20] In contrast, a German study recommended screening for gastric cancer initiating at 35 years of age based on the high frequency of this malignancy in a large cohort of Lynch syndrome families.[33] Because this study shows a considerable lifetime risk of developing gastric cancer in Lynch syndrome, surveillance of gastric cancer for MLH1 and MSH2 mutation carriers should be considered, regardless of family history. Our data demonstrate that gastric cancer was diagnosed at older than 45 years of age in the majority of gastric cancer patients. Therefore, we suggest that surveillance of gastric cancer start at 45 years of age. This is in contrast to previous studies that recommended surveillance of gastric cancer initiating at 30–35 years of age.[5] and [33] However, because of the considerable burden of surveillance for Lynch syndrome patients, critical reappraisal of starting surveillance is necessary.

Although this study demonstrates that surveillance of gastric cancer should be considered in Lynch syndrome mutation carriers, no data on large cohorts of mutation carriers support the usefulness of upper gastrointestinal endoscopy in Lynch syndrome at present. In addition, the histopathological pathway of gastric cancer in Lynch syndrome mutation carriers remains largely unclear. For these reasons, large prospective studies on these topics are necessary before any evidence-based endoscopic recommendations can be provided. Given this paucity of literature data and the difficulty obtaining lifetime gastric cancer risks in a larger cohort of Lynch patients, our suggestions seem a first step toward gastric cancer surveillance guidelines.

In conclusion, relative and absolute risks of developing gastric cancer in Lynch syndrome are substantial, particularly for patients with an MLH1 or MSH2 mutation. Family history for gastric cancer is a poor indicator for individual risk among Lynch syndrome patients. Upper gastrointestinal surveillance endoscopy should therefore be considered, regardless of family history.

## REFERENCES


1 Lynch HT, Smyrk TC, Watson P, *et al.* Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: an updated review. *Gastroenterology* 1993;104:1535-49.

2 Vasen HF. Review article: The Lynch syndrome (hereditary nonpolyposis colorectal cancer). *Aliment Pharmacol Ther* 2007;26 Suppl 2:113-26.

3 Peltomaki P, Vasen H. Mutations associated with HNPCC predisposition -- Update of ICG-HNPCC/INSiGHT mutation database. *Dis Markers* 2004;20:269-76.

4 Koornstra JJ, Mourits MJ, Sijmons RH, *et al.* Management of extracolonic tumours in patients with Lynch syndrome. *Lancet Oncol* 2009;10:400-8.

5 Vasen HF, Moslein G, Alonso A, *et al.* Guidelines for the clinical management of Lynch syndrome (hereditary non-polyposis cancer). *J Med Genet* 2007;44:353-62.

6 Watson P, Lynch HT. The tumor spectrum in HNPCC. *Anticancer Res* 1994;14:1635-9.

7 Watson P, Lynch HT. Extracolonic cancer in hereditary nonpolyposis colorectal cancer. *Cancer* 1993;71:677-85.

8 Aarnio M, Salovaara R, Aaltonen LA, *et al.* Features of gastric cancer in hereditary non-polyposis colorectal cancer syndrome. *Int J Cancer* 1997;74:551-5.

9 Maul JS, Warner NR, Kuwada SK, *et al.* Extracolonic cancers associated with hereditary nonpolyposis colorectal cancer in the Utah Population Database. *Am J Gastroenterol* 2006;101:1591-6.

10 Bermejo JL, Eng C, Hemminki K. Cancer characteristics in Swedish families fulfilling criteria for hereditary nonpolyposis colorectal cancer. *Gastroenterology* 2005;129:1889-99.

11 Warthin AS. Heredity with reference to carcinoma. *Arch Intern Med* 1913;9:279-96.

12 Vasen HF, den Hartog Jager FC, Menko FH, *et al.* Screening for hereditary non-polyposis colorectal cancer: a study of 22 kindreds in The Netherlands. *Am J Med* 1989;86:278-81.

13 Breslow N, Day N. Statistical Methods in Cancer Research: Volume II: The Design and Analysis of Cohort Studies. *IARC Sci Publ* 1987;82:1-406.

14 Lauren P. The Two Histological Main Types of Gastric Carcinoma: Diffuse and So-Called Intestinal-Type Carcinoma. an Attempt at a Histo-Clinical Classification. *Acta Pathol Microbiol Scand* 1965;64:31-49.

15 Lynch HT, Krush AJ. Cancer family "G" revisited: 1895-1970. *Cancer* 1971;27:1505-11.

16 Lynch HT, Lynch JF. Lynch syndrome: history and current status. *Dis Markers* 2004;20:181-98.

17 www.ikcnet.nl. 2006.

18 Park YJ, Shin KH, Park JG. Risk of gastric cancer in hereditary nonpolyposis colorectal cancer in Korea. *Clin Cancer Res* 2000;6:2994-8.

19 Park JG, Park YJ, Wijnen JT, *et al.* Gene-environment interaction in hereditary nonpolyposis colorectal cancer with implications for diagnosis and genetic testing. *Int J Cancer* 1999;82:516-9.

20 Aarnio M, Sankila R, Pukkala E, *et al.* Cancer risk in mutation carriers of DNA-mismatch-repair genes. *Int J Cancer* 1999;81:214-8.

21 Watson P, Vasen HF, Mecklin JP, *et al.* The risk of extra-colonic, extra-endometrial cancer in the Lynch syndrome. *Int J Cancer* 2008;123:444-9.

22 Jager AC, Bisgaard ML, Myrhoj T, *et al.* Reduced frequency of extracolonic cancers in hereditary nonpolyposis colorectal cancer families with monoallelic hMLH1 expression. *Am J Hum Genet* 1997;61:129-38.

23 Vasen HF, Stormorken A, Menko FH*, et al.* MSH2 mutation carriers are at higher risk of cancer than MLH1 mutation carriers: a study of hereditary nonpolyposis colorectal cancer families. *J Clin Oncol* 2001;19:4074-80.

24 Geary J, Sasieni P, Houlston R*, et al.* Gene-related cancer spectrum in families with hereditary non-polyposis colorectal cancer (HNPCC). *Fam Cancer* 2008;7:163-72.

25 Kastrinos F, Stoffel EM, Balmana J*, et al.* Phenotype comparison of MLH1 and MSH2 mutation carriers in a cohort of 1,914 individuals undergoing clinical genetic testing in the United States. *Cancer Epidemiol Biomarkers Prev* 2008;17:2044-51.

26 Wijnen J, de Leeuw W, Vasen H*, et al.* Familial endometrial cancer in female carriers of MSH6 germline mutations. *Nat Genet* 1999;23:142-4.

27 Wu Y, Berends MJ, Mensink RG*, et al.* Association of hereditary nonpolyposis colorectal cancer-related tumors displaying low microsatellite instability with MSH6 germline mutations. *Am J Hum Genet* 1999;65:1291-8.

28 ten Kate GL, Kleibeuker JH, Nagengast FM*, et al.* Is surveillance of the small bowel indicated for Lynch syndrome families? *Gut* 2007;56:1198-201.

29 Hendriks YM, de Jong AE, Morreau H*, et al.* Diagnostic approach and management of Lynch syndrome (hereditary nonpolyposis colorectal carcinoma): a guide for clinicians. *CA Cancer J Clin* 2006;56:213-25.

30 Lindor NM, Petersen GM, Hadley DW*, et al.* Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: a systematic review. *Jama* 2006;296:1507-17.

31 Ziogas A, Anton-Culver H. Validation of family history data in cancer family registries. *Am J Prev Med* 2003;24:190-8.

32 Gylling A, Abdel-Rahman WM, Juhola M*, et al.* Is gastric cancer part of the tumour spectrum of hereditary non-polyposis colorectal cancer? A molecular genetic study. *Gut* 2007;56:926-33.

33 Goecke T, Schulmann K, Engel C*, et al.* Genotype-phenotype comparison of German MLH1 and MSH2 mutation carriers clinically affected with Lynch syndrome: a report by the German HNPCC Consortium. *J Clin Oncol* 2006;24:4285-92.

# Chapter 9

## Increased risk of esophageal squamous cell carcinoma in patients with gastric atrophy: independent of the severity of atrophic changes

Annemarie C. de Vries [1], Lisette G. Capelle [1], Caspar W.N. Looman [2], Mark van Blankenstein [1], Nicole C.T. van Grieken [3], Mariël K. Casparie [4], Gerrit A. Meijer [2], and Ernst J. Kuipers [1, 5]

Departments of [1]Gastroenterology and Hepatology, [2]Public Health and [5]Internal Medicine Erasmus MC University Medical Center, Rotterdam, [3]Department of Pathology, VU University Medical Center, Amsterdam, [4]Prismant, Utrecht, The Netherlands

*International Journal of Cancer; 2009;124(9):2135-8*

## ABSTRACT

An association between gastric atrophy and esophageal squamous cell carcinomas (ESCC) has been described. However, the mechanism of this association is unknown. In this study, we aimed to examine this relationship in a cohort of patients with varying grades of gastric atrophy to increase the understanding about the causality of the association. Patients diagnosed with gastric atrophy between 1991 and 2005 were identified in the Dutch nationwide histopathology registry (PALGA). The incidence of ESCC and, presumably unrelated, small cell lung carcinomas (SCLC) observed in these patients was compared with that in the general Dutch population. Relative risks (RRs) and 95% confidence intervals were calculated by a Poisson model. At baseline histological examination, 97,728 patients were diagnosed with gastric atrophy, of whom 23,278 with atrophic gastritis, 65,934 with intestinal metaplasia and 8,516 with dysplasia. During follow-up, 126 patients were diagnosed with ESCC and 263 with SCLC (overall rates 0.19, respectively 0.39/1,000 person-years at risk). Compared with the general Dutch population, patients with gastric atrophy ran a RR of developing ESCC of 2.2 [95% CI 1.8-2.6] and of SCLC of 1.8 [95% CI 1.6-2.1]. The risk of ESCC did not increase with increasing severity of gastric atrophy ($p = 0.90$). In conclusion, this study found an association between gastric atrophy and both ESCC and SCLC, but the risk of ESCC did not increase with the severity of gastric atrophy. Therefore, a causal relationship seems unlikely. Confounding factors, such as smoking, may explain both associations.

## INTRODUCTION

Chronic *Helicobacter pylori* infection has been widely accepted as a predisposing condition for a number of gastric and duodenal disorders, such as peptic ulcer disease, MALT lymphoma and gastric cancer.[1] However, over the past years, interest has been directed toward the potential role of *H. pylori* infection in the etiology of esophageal diseases.[2] This new focus has emerged from epidemiological studies demonstrating a negative association between *H. pylori* infection and gastro-esophageal reflux disease (GERD) and its related complications, in particular Barrett's esophagus and esophageal adenocarcinoma.[3-6]

In addition, recent studies have demonstrated an elevated risk of esophageal squamous cell carcinomas (ESCC) in patients with atrophic changes of the gastric mucosa.[3,7-10] A hypothesis explaining this unexpected association is, however, lacking.[11] Confounding by joint risk factors such as lifestyle was not observed in case-control studies, thus suggesting a direct causal relationship between both conditions.[3,9] A causal relationship would strengthen the importance of *H. pylori* eradication in the prevention of upper gastro-intestinal malignancies. In case causality exists, the magnitude of the association would be expected to increase with the severity of gastric atrophy. On the other hand, were the association between gastric atrophy and ESCC based on confounding by shared risk factors, similar associations would be expected between gastric atrophy and other carcinomas with the same risk factors. An obvious candidate shared risk factor for the development of both gastric atrophy and ESCC is smoking.[12-14]

To examine the existence of a causal relationship between ESCC and gastric atrophy, we investigated the correlation between the severity of gastric atrophy and risk of ESCC within a large cohort of patients with varying histological stages of gastric atrophy, *i.e.* atrophic gastritis, intestinal metaplasia and dysplasia. The cascade from chronic *H. pylori* gastritis *via* atrophic gastritis, intestinal metaplasia, dysplasia toward gastric cancer has been widely accepted.[15] In line with this cascade, patients with intestinal metaplasia or dysplasia have been demonstrated to suffer from more extensive atrophic changes of the gastric epithelium when compared with patients with only atrophic gastritis.[16] In addition, we also investigated the risk of small cell lung carcinoma (SCLC) in the same study cohort, as this tumor is anatomically unrelated to the gastric and esophageal conditions and is known to be strongly associated with smoking.[17]

## MATERIAL AND METHODS

### *Histopathology database*

All histo- and cytopathology reports in The Netherlands are collected in a national archive (PALGA database), which since 1991 has had nationwide coverage.[18] Patients in this database are identified by date of birth, gender and the first 4 characters of their family name. Every record in the database contains a summary of a pathology report and diagnostic codes similar to the systematized nomenclature of medicine (SNOMED) classification of the College of American Pathologists.[19] The diagnostic code contains a term indicating the anatomical location, type of sample and a morphological term describing the finding, *e.g.* ´stomach and biopsy and intestinal metaplasia´. Details with regard to the number and intragastric location of biopsies and presence of *H. pylori* are not uniformly registered. After a report has been coded, it is submitted online to the central database. This study was based on data recorded in the PALGA database between 1991 and 2006. The following items were made available for each report: gender, date of birth, date of pathology review, summary text and diagnostic code.

### *Patient selection*

All patients with a first histologically confirmed diagnosis of gastric atrophy, *i.e.* atrophic gastritis, intestinal metaplasia and dysplasia, between 1991 and 2005 were identified in the database which had complete nationwide coverage since 1991. The most severe stage of gastric atrophy at baseline was evaluated as initial diagnosis. This meant that patients with atrophic gastritis without a diagnosis of concomitant intestinal metaplasia were classified as having atrophic gastritis, patients with atrophic gastritis and intestinal metaplasia as intestinal metaplasia and patients with gastric dysplasia as dysplasia.

As far as could be determined from the database, all patients who had undergone gastric or esophageal surgery, or had been diagnosed with an esophageal or gastric malignancy prior to, or simultaneously with the first diagnosis of a premalignant gastric lesion, were excluded from analysis.

### *Statistical analysis*

The incidences of ESCC and SCLC in the cohort of patients with gastric atrophy were calculated on the basis of the total number of ESCC and SCLC registered in the PALGA database within the cohort in relationship to the number of person-years at risk. The relative risk (RR) of ESCC and SCLC in patients with premalignant lesions of the gastric mucosa was then calculated by comparing these incidences with those for ESCC and SCLC within the general Dutch

population from 1991 until 2006. Unless an autopsy had been performed, the date of death of patients registered in the PALGA database is not recorded. Therefore, censoring because of death was imputed to evaluate the number of person-years at risk for all patients that did not develop esophageal or gastric cancer during follow-up, using survival data from the general Dutch population (Dutch Cancer Registry, personal communication, October 2007). The incidence of ESCC and SCLC in the general Dutch population were calculated on the basis of the total number of ESCC and SCLC registered in the PALGA database and the midyear Dutch population.[20] As less than 1% of all ESCC within the general Dutch population occur in patients aged below 40 years, RRs were only calculated for patients aged over 40 years.[21] The size of and incidence within the general Dutch population was corrected for the number of and incidence of ESCC and SCLC within patients with gastric atrophy. To explore the presence of selection bias, ESCC risk was calculated for the first year of follow-up, between 1 and 4 years follow-up and after more than 4 years follow-up after the initial diagnosis of gastric atrophy. The RRs and 95% confidence intervals (CIs) were calculated by a Poisson model, corrected for age categories, gender and calendar year. Comparisons of RRs between different groups were also calculated with the Poisson model.

## RESULTS

The study cohort consisted of 97,728 patients (49,739 men/47,989 women) with a first histological diagnosis of gastric atrophy registered between 1991 and 2005. It comprised atrophic gastritis in 23,278 (24%) patients, intestinal metaplasia in 65,934 (67%) patients and dysplasia in 8,516 (9%) patients (Table I). Overall, mean age at diagnosis was 63.5 years (SD 15.6). Data on the incidence of gastric atrophy over the study period have been published previously.[22]

**Table 1.** Baseline characteristics of our study population

| | Total | Atrophic gastritis | Intestinal metaplasia | Dysplasia |
|---|---|---|---|---|
| Number of patients (n) (%) | 97 728 | 23 278 (24%) | 65 934 (67%) | 8 516 (9%) |
| Male/ Female | 49 739/ 47 989 (51/49) | 10 527/ 12 751 (45/55) | 34 573/ 31 361 (52/48) | 4 639/ 3 877 (54/46) |
| Age (years) | | | | |
| mean | 63.5 | 59.2 | 64.7 | 66.7 |
| 25th-75th percentile | 53.2- 75.5 | 46.6- 73.4 | 55.0- 75.9 | 57.3- 77.6 |

### *Esophageal squamous cell carcinomas*

Between 1991 and 2006, ESCC was diagnosed in 126 patients (77 men/49 women) from the cohort at a mean age of 68.7 years (SD 11.3). The rate of developing ESCC was 0.19/1,000 person-years at risk in patients older than 40 years.

For all patients with gastric atrophy, the long-term RR of ESCC was 1.98 [95% CI 1.58-2.48] in male patients and 2.52 [95% CI 1.90-3.34] in female patients when compared with the general Dutch population aged over 40 years (Table II). The overall RR of ESCC within the first year of follow-up after the diagnosis of gastric atrophy was significantly higher when compared with 1-4 years or more than 4 years follow-up (RR 5.99 [95% CI 4.48-8.01], respectively RR 1.57 [95% CI 1.11-1.21] and RR 1.53 [95% CI 1.16-2.03]) ($p < 0.001$). In patients with atrophic gastritis as the most severe diagnosis at baseline, the RR was 1.90 (95% CI 1.15-3.16] for men and 2.84 [95% CI 1.71-4.72] for women. In patients with intestinal metaplasia and dysplasia the RRs were respectively 2.06 [95% CI 1.60-2.68] in men and 2.16 [95% CI 1.49-3.14] in women, and 1.53 [95% CI 0.69-3.40] in men and 4.10 [95% CI 1.96-8.56] in women. Therefore, for both men and women, the risk of ESCC did not increase with the severity of premalignant gastric lesions at baseline ($p = 0.82$, respectively $p = 0.83$). Similarly, no significant difference was demonstrated for the risk of ESCC between different histological diagnoses at baseline within the first year of follow-up, 1-4 years follow-up, or more than 4 years follow-up ($p = 0.69$, respectively $p = 0.14$ and $p = 0.11$).

**Table 2.** Relative risk of esophageal squamous cell carcinomas in patients with pre-malignant gastric lesions in comparison to the general Dutch population, corrected for age and sex.

| | Number of cases | Relative risk | 95% CI | Number of cases SCLC | Relative risk SCLC | 95% CI |
|---|---|---|---|---|---|---|
| Overall | 126 | 2.16 | 1.81-2.57 | 263 | 1.84 | 1.63-2.07 |
| Sex | | | | | | |
| - Male | 77 | 1.98 | 1.58-2.48 | 182 | 1.64 | 1.41-1.90 |
| - Female | 49 | 2.52 | 1.90-3.34 | 81 | 2.55 | 2.05-3.17 |
| Age at baseline | | | | | | |
| - 40-54 years | 19 | 3.56 | 2.27-5.59 | 17 | 2.97 | 1.85-4.77 |
| - 55-69 years | 40 | 1.77 | 1.30-2.42 | 119 | 2.32 | 1.93-2.78 |
| - ≥ 70 years | 67 | 2.19 | 1.72-2.80 | 127 | 1.47 | 1.23-1.75 |
| Most severe pre-malignant lesion at baseline | | | | | | |
| - Atrophic gastritis | 30 | 2.28 | 1.59-3.26 | 36 | 1.19 | 0.87-1.63 |
| - Intestinal metaplasia | 83 | 2.09 | 1.69-2.59 | 200 | 2.02 | 1.76-2.32 |
| - Dysplasia | 13 | 2.31 | 1.35-3.97 | 27 | 1.88 | 1.29-2.74 |

### *Small cell lung carcinomas*

In total, 263 patients (182 men/81 women) from the cohort were diagnosed with SCLC at a mean age of 69.0 years (SD 8.5). The rate of developing SCLC was 0.39/1,000 person-years at risk in patients older than 40 years. For all patients with gastric atrophy, the RR of SCLC was 1.64 [95% CI 1.41-1.90] in male patients and 2.55 [95% CI 2.05-3.17] in female patients when compared with the general Dutch population. Here, again there was no relationship between the severity of gastric atrophy and the risk of development of SCLC (Table II).

## DISCUSSION

This large, nationwide study confirms a positive association between gastric atrophy and the risk of ESCC. Our findings showed an overall RR of 2.2 for the development of ESCC in patients with gastric atrophy. However, the risk of ESCC in our population did not increase with the severity of gastric atrophy, with RRs of 2.3 for atrophic gastritis, 2.1 for intestinal metaplasia and 2.3 for dysplasia being observed.

There were considerable variations in the magnitude of the association between gastric atrophy and ESCC observed in previous studies from Sweden and Japan and in our study.[3,7-9] These differences may have resulted from the fact that all studies used different study populations and detection methods of gastric atrophy. In the Swedish studies the diagnosis of gastric atrophy was based on surrogate markers, *i.e.* either clinically diagnosed pernicious anemia, gastric ulcer disease or pepsinogen-I serology, resulting in elevated risks of respectively 3.3, 1.8 and 4.3 times for the development of ESCC when compared with the general population. In the Japanese study, the diagnosis of gastric atrophy was based on both pepsinogen-I serology and histology, resulting in elevated risks of 8.2 and 4.2, respectively. In contrast, instead of employing such surrogate markers, our study was able to estimate the ESCC risk within a population with histologically confirmed gastric atrophy. The selection of patients aged above 40 years has not influenced the generalizability of our observations to the whole population, as the incidence of ESCC is extremely low under this age both in our cohort (none of the cases) and in the general Dutch population (considerably >1% of all ESCC cases). In addition, our study shows that the risk of developing ESCC is especially high within the first year of follow-up. The high number of ESCC diagnoses shortly after the diagnosis of gastric atrophy suggests the presence of selection bias, as this could for instance have resulted from overlooking an incipient cancer or sampling error during the first endoscopy. The presence of selection bias has probably overestimated the overall RR of ESCC in this and previous studies.

Although this risk of developing ESCC was significantly higher in patients with gastric atrophy than in the general Dutch population, this association lacks clinical relevance, as the magnitude of the association was far too small to direct surveillance practices. Nevertheless, this association could provide important insights into the pathogenesis of both conditions.

The positive association between ESCC and gastric atrophy is not easily explained, it could either be causal or the result of confounding risk factors involving both conditions. Previous case-control studies from Sweden and Japan reported gastric atrophy to increase the risk of ESCC independently of patently obvious confounding risk factors, such as smoking.[3,9,12] In addition, the Japanese investigators observed the ESCC risk to correlate positively to the severity of gastric atrophy.[9] Possible mechanisms for a causal relationship were suggested, for instance that achlorhydria in patients with gastric atrophy may constitute an intragastric environment favoring bacterial overgrowth and bacterial n-nitrosation resulting in an increased exposure of the esophageal mucosa to carcinogenic endogenous nitrosamines.[11]

As patients with intestinal metaplasia have more extensive and generally longer existing atrophic changes of the gastric mucosa when compared with patients with merely atrophic gastritis, an increased formation of carcinogenic mediators, and thus a higher incidence of ESCC may be expected in patients with intestinal metaplasia.

Our findings in this large cohort study of patients with histologically confirmed cases of gastric atrophy, however, contradict these observations. The absence of any association between the severity of gastric atrophy and the risk of ESCC undermines the presence of a causal relationship between both conditions. The absence of a causal relationship is further supported by the finding that within different intervals of follow-up no significant ESCC risk was demonstrated between patients with atrophic gastritis, intestinal metaplasia or dysplasia. As no causal relationship was demonstrated, *H. pylori* eradication is unlikely to prevent the development of ESCC. Moreover, the demonstration of a similar association between gastric atrophy and SCLC in this study not only demonstrates the spuriousness of the previously assumed relationship but also points to joint causal risk factors for all 3 conditions, the most prominent of which is obviously smoking. The discrepancy between our data and those of previous studies is probably explained by the relatively small number of patients included in previous studies. For example, in the study from Japan only 29 patients with intestinal metaplasia were included.

Nevertheless, other explanations for the positive association between gastric atrophy and ESCC are worth exploring. It may well be that both conditions do share genetically determined pathogenetic mechanisms facilitating a similar destructive process, which damages both the gastric and esophageal epithelium, for instance *via* inflammatory response or defective DNA repair.[23-25] Prospective studies into these mechanisms may elucidate such an association. In addition, the observed association between gastric atrophy and SCLC may result from an unidentified interaction between the upper gastrointestinal tract and the lung. Such an association has been described, as for instance an increased prevalence of asthma in subjects with gastro-esophageal reflux.[26] Moreover, the production of carcinogenic nitrosamines in the atrophic stomach may theoretically cause lung cancer *via* a haematogenous route.[27] These hypotheses are also worth exploring in future research.

Strengths of our study are the nationwide selection of individuals and the large number of patients with histological confirmed diagnoses included in this study. Nevertheless, in spite of its large size, the selection of our study population will not have been complete, as not all subjects with gastric atrophy in the general population have undergone endoscopy with biopsy sampling and thus been diagnosed. Therefore, the general population that was used as control-group in this study would certainly include patients with undetected gastric atrophy, which could have resulted in underestimating the true RRs of the association between both conditions. Second, it was impossible to calculate ESCC risk for different intragastric locations of gastric atrophy, as the intragastric location of biopsies is not uniformly registered in the PALGA database. However, it has been recognized for long that premalignant

gastric lesions occur most commonly in the antrum and incisura angularis. Subsequently, these lesions spread along the lesser curvature to the proximal stomach and at the same time increase in severity. Biopsies from the antrum are commonly obtained during routine upper gastrointestinal endoscopy. Therefore, the diagnoses in this study most likely reflect the most severe gastric lesions at baseline. As patients with intestinal metaplasia or dysplasia have generally more extensive and longer existing atrophic changes of the gastric mucosa, they also demonstrate a higher prevalence of fundic atrophy. Therefore, no difference in the risk of ESCC and gastric atrophy between patients with distal gastric atrophy and patients with fundic atrophy was demonstrated in this study. Third, as patients were treated in all hospitals throughout the country, differences in histological assessment cannot be excluded. However, the large number of patients in this study most likely compensates for these variations. Fourth, we lack information on possible confounding risk factors. However, as we demonstrated an association between gastric atrophy and SCLC, we can presume that these confounders are present. Finally, we used imputation of survival estimates to calculate the RRs. The imputation of survival estimates was based on the assumption that patients with gastric atrophy had a life expectancy similar to the general population. However, as they may well suffer from increased comorbidity and mortality, this assumption could have led to an overestimation of the cohort at risk, and consequently, an underestimation of its RR of developing ESCC and SCLC.[28] Nevertheless, we think that these comorbidities have only slightly influenced the reported RRs, as the large size of this study presumably compensated these inaccuracies and their limited effect on overall mortality.

In conclusion, although this study confirms a positive association between gastric atrophy and ESCC, the risk of ESCC does not increase in parallel with the increasing severity of gastric atrophy. Therefore, a causal relationship between gastric atrophy and ESCC seems unlikely. Moreover, as a similar association was demonstrated between gastric atrophy and the anatomically unrelated SCLC, these associations are best explained by confounding factors, such as smoking.

## REFERENCES


1 Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of *Helicobacter pylori* infection. *Clin Microbiol Rev* 2006; 19: 449-90.

2 Malfertheiner P, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, Kuipers EJ. Current concepts in the management of *Helicobacter pylori* infection: the Maastricht III Consensus Report. *Gut* 2007; 56: 772-81

3 Ye W, Held M, Lagergren J, Engstrand L, Blot WJ, McLaughlin JK, Nyren O. *Helicobacter pylori* infection and gastric atrophy: risk of adenocarcinoma and squamous-cell carcinoma of the esophagus and adenocarcinoma of the gastric cardia. *J Natl Cancer Inst* 2004; 96: 388-96

4 Wu AH, Crabtree JE, Bernstein L, Hawtin P, Cockburn M, Tseng CC, Forman D. Role of *Helicobacter pylori* CagA+ strains and risk of adenocarcinoma of the stomach and esophagus. *Int J Cancer* 2003; 103: 815-21

5 Chow WH, Blaser MJ, Blot WJ, Gammon MD, Vaughan TL, Risch HA, Perez-Perez GI, Schoenberg JB, Stanford JL, Rotterdam H, West AB, Fraumeni JF, Jr. An inverse relation between cagA+ strains of *Helicobacter pylori* infection and risk of esophageal and gastric cardia adenocarcinoma. *Cancer Res* 1998; 58: 588-90

6 Vicari JJ, Peek RM, Falk GW, Goldblum JR, Easley KA, Schnell J, Perez-Perez GI, Halter SA, Rice TW, Blaser MJ, Richter JE. The seroprevalence of cagA-positive *Helicobacter pylori* strains in the spectrum of gastroesophageal reflux disease. *Gastroenterology* 1998; 115: 50-7

7 Bahmanyar S, Zendehdel K, Nyren O, Ye W. Risk of oesophageal cancer by histology among patients hospitalised for gastroduodenal ulcers. *Gut* 2007; 56: 464-8

8 Ye W, Nyren O Risk of cancers of the oesophagus and stomach by histology or subsite in patients hospitalised for pernicious anaemia. *Gut* 2003; 52: 938-41

9 Iijima K, Koike T, Abe Y, Inomata Y, Sekine H, Imatani A, Nakaya N, Ohara S, Shimosegawa T. Extensive gastric atrophy: an increased risk factor for superficial esophageal squamous cell carcinoma in Japan. *Am J Gastroenterol* 2007; 102: 1603-9

10 Rakic S, Dunjic MS, Pesko P, Milicevic M. Atrophic chronic gastritis in patients with epidermoid carcinoma of the esophagus. *J Clin Gastroenterol* 1993; 17: 84

11 McColl KE. *Helicobacter pylori* and oesophageal cancer - not always protective. *Gut* 2007; 56: 457-9

12 Kneller RW, You WC, Chang YS, Liu WD, Zhang L, Zhao L, Xu GW, Fraumeni JF, Jr, Blot WJ. Cigarette smoking and other risk factors for progression of precancerous stomach lesions. *J Natl Cancer Inst* 1992; 84: 1261-6

13 Russo A, Maconi G, Spinelli P, Felice GD, Eboli M, Andreola S, Ravagnani F, Settesoldi D, Ferrari D, Lombardo C, Bertario L. Effect of lifestyle, smoking, and diet on development of intestinal metaplasia in *H. pylori*-positive subjects. *Am J Gastroenterol* 2001; 96: 1402-8

14 Engel LS, Chow WH, Vaughan TL, Gammon MD, Risch HA, Stanford JL, Schoenberg JB, Mayne ST, Dubrow R, Rotterdam H, West AB, Blaser M, et al. Population attributable risks of esophageal and gastric cancers. *J Natl Cancer Inst* 2003; 95: 1404-13

15 Kuipers EJ, Uyterlinde AM, Pena AS, Roosendaal R, Pals G, Nelis GF, Festen HP, Meuwissen SG. Long-term sequelae of *Helicobacter pylori* gastritis. *Lancet* 1995; 345: 1525-8

16 Guarner J, Herrera-Goepfert R, Mohar A, Sanchez L, Halperin D, Ley C, Parsonnet J. Gastric atrophy and extent of intestinal metaplasia in a cohort of *Helicobacter pylori*-infected patients. *Hum Pathol* 2001; 32: 31-5

17 Jackman DM, Johnson BE. Small-cell lung cancer. *Lancet* 2005; 366: 1385-96

18 Casparie M, Tiebosch T, Burger G, Blauwgeers H, van de Pol A, van Krieken J, Meijer G. Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. *Cell Oncol* 2007; 29: 19-24
19 Cote RA,Robboy S. Progress in medical information management. Systematized nomenclature of medicine (SNOMED). *JAMA* 1980; 243: 756-62
20 Statistics Netherlands. [Internet] 2008. [cited June 2008] Available at: www.cbs.nl. 2007
21 [Internet] 2008 [cited June 2008]. Available at: www.ikcnet.nl. 2003
22 de Vries AC, Meijer GA, Looman CW, Casparie MK, Hansen BE, van Grieken NC, Kuipers EJ. Epidemiological trends of pre-malignant gastric lesions: a long-term nationwide study in the Netherlands. *Gut* 2007; 56: 1665-70
23 Hold GL, Rabkin CS, Chow WH, Smith MG, Gammon MD, Risch HA, Vaughan TL, McColl KE, Lissowska J, Zatonski W, Schoenberg JB, Blot WJ, et al. A functional polymorphism of toll-like receptor 4 gene increases risk of gastric carcinoma and its precursors. *Gastroenterology* 2007; 132: 905-12
24 El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000; 404: 398-402
25 Moons LM, Kuipers EJ, Rygiel AM, Groothuismink AZ, Geldof H, Bode WA, Krishnadath KK, Bergman JJ, van Vliet AH, Siersema PD, Kusters JG. COX-2 CA-haplotype is a risk factor for the development of esophageal adenocarcinoma. *Am J Gastroenterol* 2007; 102: 2373-9
26 Nordenstedt H, Nilsson M, Johansson S, Wallander MA, Johnsen R, Hveem K, Lagergren J. The relation between gastroesophageal reflux and respiratory symptoms in a population-based study: the Nord-Trøndelag health survey. *Chest* 2006; 129: 1051-6
27 Kitamura Y, Umemura T, Kanki K, Ishii Y, Kuroiwa Y, Masegi T, Nishikawa A, Hirose M. Lung as a new target in rats of 2-amino-3-methylimidazo[4, 5-f]quinoline carcinogenesis: results of a two-stage model initiated with N-bis(2-hydroxypropyl)nitrosamine. *Cancer Sci* 2006; 97: 368-73
28 de Vries AC, van Grieken NC, Looman CW, Casparie MK, de Vries E, Meijer GA, Kuipers EJ. Gastric cancer risk in patients with premalignant gastric lesions: a nationwide cohort study in the Netherlands. *Gastroenterology* 2008; 134: 945-52

# Chapter 10

## General discussion and conclusions

## INTRODUCTION

*Helicobacter pylori* infection shows a high prevalence worldwide.[1] In the past decades, extensive research demonstrated that gastric colonization with *H. pylori* is always associated with chronic active gastritis, which can result in a variety of diseases such as peptic ulceration, pre-malignant lesions of the gastric mucosa, gastric adenocarcinoma, and gastric MALT lymphoma.[2] Despite the fact that only a small percentage of *H. pylori*–positive subjects eventually develop gastric cancer, this condition remains due to the high prevalence of *H. pylori* a major health problem as second leading cause of cancer related death worldwide.[3,4] Gastric adenocarcinoma is often asymptomatic until advanced stage of disease when treatment options are limited. As a consequence 5 year survival of gastric cancer is low.[5] Data on epidemiology and gastric cancer risk of pre-malignant conditions, such as gastric MALT lymphoma, pre-malignant gastric lesions and Lynch syndrome can provide new insights in early detection and treatment of gastric cancer and may eventually lead to a reduction in gastric cancer mortality. Therefore, in this thesis we aimed to address the epidemiology of gastric cancer in MALT lymphoma patients and Lynch syndrome mutation carriers. Furthermore, the gastric cancer risk and screening and surveillance options for patients with gastric MALT lymphoma, pre-malignant gastric lesions and Lynch syndrome mutation carriers were investigated.

## MAIN FINDINGS

### *Genetics*

Previous studies have been investigating *H. pylori* susceptibility for years. Although the role of environmental factors in *H. pylori* susceptibility has been widely accepted, the association between *H. pylori* susceptibility and genetic factors remained less clear. A previous study demonstrated that the concordance rate for *H. pylori* in monozygotic twins was significantly higher than for dizygotic twins.[6] However, specific associations between *H. pylori* and genetic polymorphisms were unknown. For these reasons we performed a pilot genome wide association study among 1005 subjects. This study showed that two loci on chromosome 2 and 11 have considerable genome wide evidence for an association with *H. pylori* susceptibility (**Chapter 2**). These newly discovered loci may provide novel insights in the role of genetic factors in *H. pylori* susceptibility. However, our findings await confirmation in larger population with combination of genome wide results, and the function of these two loci needs to be further investigated.

## *Epidemiology of H. pylori-associated malignancies*

### *Gastric MALT lymphoma*

Similar to gastric cancer, gastric MALT lymphoma is usually asymptomatic. As a result, studies on epidemiology of gastric MALT lymphoma show a highly varying incidence within different countries, ranging from an incidence of 0.21 per 100 000 to an incidence of 13 per 100 000 people.[3,7] These differences are explained by varying *H. pylori* prevalence between the included study populations and differences in study design.[7-9] *H. pylori* has been considered a predisposing condition since the first study in 1983 demonstrated that these organisms were present in over 90% of gastric MALT lymphoma cases.[10-12] Moreover, eradication of *H. pylori* alone leads to partial or complete remission in 60-80% of patients.[10,11] Despite this strong evidence for an association between *H. pylori* and gastric MALT lymphoma, previous studies described an increasing incidence of gastric MALT lymphoma from the eighties to nineties in contrast to the declining incidence of *H. pylori* infection in these years.[12-14] Whether this was a true increase or related to a change in diagnostic criteria with the availability of new treatment options remained unclear. We therefore performed a nationwide study to evaluate incidence trends from 1991 to 2006 (**Chapter 3**). The increase in incidence of gastric MALT lymphoma between 1991 and 1997 is confirmed in this study. However, after 1997 the incidence of gastric MALT lymphoma showed a rapid decline. Explanations for the increasing incidence are the increasing interest in gastric MALT diagnosis after the discovery of the *H. pylori* association. Gastric MALT lymphoma became an infection-associated malignant disease with improved treatment options.[11,15] Moreover, improvement in diagnostic and endoscopic procedures may also have contributed to the gastric MALT lymphoma increase.[16-18] Currently, the incidence of gastric MALT lymphoma is rapidly declining in the Netherlands. This decrease is most probably explained by the decreasing *H. pylori* prevalence in Western countries similar to the incidence of other *H. pylori*-associated malignancies. Despite the decreasing incidence, accurate endoscopic and histologic reevaluation is warranted as gastric cancer risk is considerable in gastric MALT lymphoma patients.

### *Lynch syndrome*

In 1913, the first description of a family with 'cancer syndrome G' was described, by Warthin et al.[19] Dr. A. Warthin, a pathologist, described the family of his seamstress as he learned that she was distressed about the fact that she would die early from cancer, like the rest of her family. As she predicted, she died at young age of endometrial cancer.[20-22] In this family, now known as Warthin's family G, gastric cancer was a predominate type of cancer. Nowadays, we know that this 'family G' harbored mutations in mismatch repair genes. Currently, this syndrome caused by mutations in mismatch repair genes is called Lynch syndrome. The occurrence of gastric cancer decreased over the past decades in Warthin's 'family G' and in the newly discovered Lynch syndrome families. However, exact incidence trends of gastric cancer in Lynch

syndrome mutation carriers were unknown. Such knowledge is of importance to develop and target optimal prevention strategies for gastric cancer in Lynch syndrome families. In order to gain certainty on incidence trends of gastric cancer in Lynch syndrome mutation carriers, we analyzed the tumour spectrum of 236 families registered in the HNPCC registry established in Leiden.[23] With these data, we demonstrated a decrease in standardized incidence rate of gastric cancer from 4.0 to 2.0 from 1970 to 2003 (**Chapter 8**). Although, previous studies also described a declining incidence of gastric cancer in Lynch syndrome families, in our study the decline in incidence was non-significant.[21,24] This may indicate that the gastric cancer incidence is not further decreasing in the recent decades in Lynch syndrome mutation carriers.

## *Gastric cancer risk and screening and surveillance options of H. pylori-associated malignancies*

### *Gastric MALT lymphoma*

Gastric adenocarcinoma risk in patients with gastric MALT lymphoma remains highly controversial.[15,25-30] For these reasons, surveillance of patients with gastric MALT lymphoma for a diagnosis of gastric cancer is often omitted in clinical practice. To provide gastric cancer risk estimates in patients with gastric MALT lymphoma in Western countries, we calculated absolute and relative risk of developing gastric cancer in a nationwide study (**Chapter 3**). In this study, we demonstrated a six times increased risk of developing gastric cancer in patients with gastric MALT lymphoma in comparison to the general Dutch population. Furthermore, we showed that gastric cancer occurred simultaneously with gastric MALT lymphoma diagnosis in 53% of patients, and in 38% gastric cancer was diagnosed after a diagnosis of a gastric MALT lymphoma. The interval between gastric MALT lymphoma diagnosis and gastric cancer ranged between 1 and 7 years, which was similar to intervals described in previous studies.[27] In contrast to previous studies that reported complete remission of gastric MALT lymphoma after 3,5 years of follow-up, the findings in our study emphasizes the need for accurate histological and endoscopic re-evaluation of the gastric mucosa after gastric MALT lymphoma diagnosis.

Similar to previous studies, we demonstrated that a diagnosis of gastric MALT lymphoma is rare in the Netherlands, with an overall incidence of 0.4 per 100 000 per year.[3,9] This low incidence of gastric MALT lymphoma with an even smaller percentage of patients that develop gastric cancer, indicates that surveillance of all gastric MALT lymphoma should be limited to patients at high gastric cancer risk. Previous studies reported an increasing incidence of pre-malignant gastric lesions in gastric MALT lymphoma patients.[31-34] Identification of these lesions in gastric MALT patients might lead to a subpopulation at high gastric cancer risk, for which strict surveillance is necessary. For these reasons, the prevalence of pre-malignant gastric lesions was evaluated in gastric MALT lymphoma patients and in patients with diffuse large B-cell lymphomas (DLBCL), which was previously defined as 'high grade gastric MALT

lymphoma'.[35] We compared the prevalence of pre-malignant gastric lesions in patients with a subsequent diagnosis of gastric cancer and in patients without a subsequent diagnosis of gastric cancer matched for age and years of follow-up. The prevalence of pre-malignant gastric lesions was neither significantly different between patients with and without a subsequent diagnosis of gastric cancer, nor between patients with gastric MALT lymphoma and patients with diffuse large B-cell lymphomas (**Chapter 4**). Surprisingly, the prevalence of severe pre-malignant lesions such as intestinal metaplasia and dysplasia was substantial in both gastric MALT lymphoma patients as well as diffuse large B-cell lymphoma patients. These findings indicate that thoroughly scrutinizing the gastric mucosa at lymphoma diagnosis is necessary and surveillance of patients with pre-malignant lesions is warranted. Furthermore, although the link between diffuse large B-cell lymphoma and *H. pylori* infection is less clear than the association between *H. pylori* and gastric MALT lymphoma, our study provided evidence that there seems to be an association between pre-malignant gastric lesions and diffuse large B-cell lymphoma.[36] Whether this association is significant remains unclear, due to the limited number of included patients. Further research with the inclusion of a large population of patients with diffuse large B-cell lymphoma is needed to confirm such an association.

*Pre-malignant gastric lesions*

The high prevalence of gastric cancer in countries such as Japan has led to the implementation of mass nationwide screening programs. Previous studies demonstrated that serological markers can accurately detect patients with pre-malignant lesions and as a result, serological markers have been introduced as part of these screening programs in Japan.[37-40] In particular for the diagnosis of atrophic gastritis, serological testing for a combination of pepsinogens I and II, gastrin and *H. pylori* antibodies has yielded accurate results.[41-45] However, there seems still a need for serological markers that improve the efficacy of non-invasive screening for advanced pre-malignant gastric lesions. Gastric leptin is similar to pepsinogen I, produced by chief cells of the gastric mucosa.[46,47] Therefore, we hypothesized that serum leptin levels may identify patients at high gastric cancer risk (**Chapter 5**). Indeed, our study demonstrated that serum levels of leptin in combination with the established risk factors male sex, advancing age and low pepsinogen I levels can serve as a tool to detect patients at high gastric cancer risk. However, the additional value of serum leptin levels was rather limited.

The low gastric incidence in the Netherlands emphasizes the need for a selective screening program in the Netherlands limited to patients at high gastric cancer risk.[40,48-50] Therefore, a risk classification based on a combination of epidemiological, clinical and serological parameters seems an appropriate approach for screening of patients in countries with a low gastric cancer incidence, with additional endoscopic surveillance of patients with an abnormal risk profile.[40] Currently, surveillance of patients with pre-malignant gastric lesions relies on histology of biopsy specimens obtained during conventional endoscopy. However, since the

endoscopic evaluation of pre-malignant lesions showed high interobserver variability and has poor correlation to histological findings, numerous other endoscopic techniques have been developed to overcome these limitations.[51-56] Narrow-band imaging (NBI) is a promising new imaging technique and showed high diagnostic accuracy in detecting gastrointestinal lesions.[57-59] However, previous studies were primarily performed in Japan, and as a consequence the additional value of NBI in the detection of advanced pre-malignant gastric lesions in Western countries remained less clear. Therefore, we performed a prospective study of patients previously diagnosed with intestinal metaplasia or dysplasia who all underwent surveillance endoscopy with conventional white light endoscopy and narrow-band imaging (**Chapter 6**). In this study we showed a somewhat lower sensitivity of 71% for the detection of intestinal metaplasia and dysplasia with NBI compared to the previous Japanese studies that demonstrated sensitivity for the detection of intestinal metaplasia of 89% with NBI.[57] This difference is probably explained by the various training techniques for Japanese and Western gastroenterologists. Nevertheless, the sensitivity for NBI increased with 20% compared to white light endoscopy. Thus the use of NBI in the surveillance of patients with pre-malignant gastric lesions is superior to conventional white light endoscopy and should be implemented in surveillance programs for patients at high gastric cancer risk.

Previous studies demonstrated that the progression rate to gastric cancer was high for patients with dysplasia, whereas only 0.8% and 1.8% for patients with atrophic gastritis and intestinal metaplasia develop gastric cancer respectively.[48] These observations indicated that surveillance endoscopy should not be advised to all patients with atrophic gastritis and intestinal metaplasia, but should preferably be limited to patients at high gastric cancer risk.[48] Only recently a new histological staging system based on the grading of gastritis was proposed that identified a subpopulation of patients at high gastric cancer risk.[60-63] Although this system demonstrated great potential in guiding clinical decisions, the use of atrophic gastritis as the principal parameter may be its major drawback, most importantly for reasons of limited reproducibility. In contrast to atrophic gastritis, intestinal metaplasia is associated with much higher interobserver agreement.[64] For these reasons, we proposed a new staging system based on the severity and extent of intestinal metaplasia (OLGIM) instead of atrophic gastritis (**Chapter 7**). With this adaptation we firstly showed an improved reproducibility for the OLGIM staging system and thus a more consistent gastric cancer risk assessment. Secondly, with the use of the OLGIM a smaller subpopulation of patients at high risk of gastric cancer was defined. For these reasons, the use of the OLGIM staging system might lead to more feasible and cost-effective surveillance strategies for patients at risk of gastric cancer and a more consistent gastric cancer risk assessment.

*Lynch syndrome*

Besides patients with gastric MALT lymphoma and patients with pre-malignant gastric lesions, Lynch syndrome mutation carriers also seem to have an increased risk of gastric cancer development.[65,66] However, recommendations for surveillance of gastric cancer in Lynch syndrome carriers vary greatly between East and West and even within Western Countries.[67-69] For these reasons, we analyzed incidence trends and gastric cancer risk in a large cohort of Lynch syndrome mutation carriers in the Netherlands (**Chapter 8**). We demonstrated that Lynch syndrome mutation carriers are indeed at increased risk of developing gastric cancer, particularly males and carriers of an MLH1 and MSH2 mutation. None of the MSH6 mutation carriers developed gastric cancer in our cohort of patients. In line with these observations, previous studies described that MSH6 mutation carriers are at high risk of developing endometrial cancer at advancing age and no small bowel tumours were observed in these patients.[70-74] Since our study showed a considerable lifetime risk of developing gastric cancer in Lynch syndrome, surveillance of gastric cancer for MLH1 and MSH2 mutation carriers should be considered, regardless of family history. Furthermore, our data demonstrated that gastric cancer was diagnosed > 45 years of age in the majority of gastric cancer patients. Therefore, we suggested that surveillance of gastric cancer starts at 45 years of age. In addition, despite lacking evidence, previous studies recommended to target upper gastrointestinal surveillance endoscopy to those families with more than one family member with gastric cancer.[75-78] Since our study showed that the majority of cases with gastric cancer occurred as single cases within a Lynch syndrome family, we concluded that family history is a poor determinant to offer or withhold surveillance for gastric cancer in Lynch syndrome mutation carriers.

*Esophageal squamous cell carcinoma*

Finally, we examined the correlation between gastric atrophy and the risk of esophageal squamous cell carcinoma (ESCC), as previous studies described an elevated risk of developing ESCC in patients with atrophic changes of the gastric mucosa.[79,80] A causal relation would suggest that *H. pylori* eradication is likely to prevent ESCC. However, the association between gastric atrophy and ESCC could also been based on confounding by shared risk factors. Our study confirmed the positive association between gastric atrophy and ESCC with an overall relative risk of 2.2 for the development in gastric atrophy patients (**Chapter 9**). Since the risk of ESCC did not increase in parallel with increasing severity of gastric atrophy and a similar association between gastric atrophy and small cell lung cancer was demonstrated, we assumed that a joint causal risk factor for all three conditions caused the positive association, this joint causal risk factor was most likely smoking.

## CONCLUSIONS AND FUTURE DIRECTIONS

### *Genetics*

Our findings of a genome wide association between genetic factors and *H. pylori* susceptibility need confirmation in larger populations with genome wide data. We are therefore expanding our study population and exploring international collaborations for confirmation cohorts. Furthermore, as function of the underlying loci remains unclear, further basic research for these loci may provide new insights in *H. pylori* susceptibility. This knowledge may eventually lead to more consistent evidence for genetic polymorphisms that play a role in *H. pylori* susceptibility.

### *H. pylori-associated malignancies*

Other than with conditions like Barrett's esophagus or colonic adenomas, most endoscopists do not know how to manage patients with *H. pylori*-associated pre-malignant conditions. Our data show that surveillance of patients with gastric MALT lymphoma, pre-malignant gastric lesions and Lynch syndrome mutation carriers is necessary and may eventually lead to a reduction in gastric cancer mortality. Since gastric MALT lymphoma patients showed a six times increased risk for developing gastric cancer and a high prevalence of pre-malignant gastric lesions at diagnosis of gastric MALT lymphoma, endoscopic surveillance of these patients in particular during the first years after diagnosis seems highly warranted. In addition, the development of pre-malignant gastric lesions in patients with diffuse large B-cell lymphomas and the increased risk for gastric cancer in patients with 'high grade MALT lymphoma' suggest that *H. pylori* may be implicated in the etiology of this type of gastric lymphoma. Therefore, surveillance strategies aiming at the early detection of pre-malignant gastric lesions in both patients with gastric MALT lymphoma and patients with diffuse large B-cell lymphomas may eventually result in a reduction in gastric cancer mortality. Possible improvement of these strategies should be based on the severity and extent of these gastric lesions assessed by the OLGIM staging system, stratification for *H. pylori* status and translocation status in gastric lymphoma patients. However, long-term prospective studies are necessary to accomplish these remaining questions and to decrease gastric cancer development in gastric MALT lymphoma patients.

For pre-malignant gastric lesions, we showed that serum leptin levels have an additional value in detecting intestinal metaplasia and dysplasia of the gastric mucosa. These findings strengthen the fact that screening for serological markers may identify a subpopulation of patients at high risk of gastric cancer. A nationwide screening program in countries with a low gastric cancer prevalence seems however not worthwhile. For these reasons, future large

population based screening programs are necessary to investigate cost-effectiveness and burden of patients in countries with low incidence of gastric cancer.

Similarly, surveillance of patients with pre-malignant gastric lesions remains controversial in Western countries. Although previous studies demonstrated a high risk of gastric cancer development, clear guidelines for these patients are still lacking. Our data showed that narrow-band imaging of the gastric mucosa is of additional value in particularly in a surveillance setting. In addition, magnification endoscopy demonstrated best results for the detection of atrophic gastritis and gastric cancer. A combination of NBI and magnification endoscopy is likely to serve as an accurate alternative for conventional white light endoscopy. Cost-effectiveness and evaluation of NBI in combination with magnification awaits therefore confirmation in large prospective studies that evaluate long-term outcome of surveillance of patients with pre-malignant gastric lesions. In addition, the role of the OLGIM staging system proposed in this thesis seems an easy tool to identify patients with advanced risk of gastric cancer development. However, only a limited number of patients with gastric cancer were included in our study, consequently, the gastric cancer risk of patients with a high risk score according to the OLGIM staging system needs to be further evaluated.

Finally, Lynch syndrome mutation carriers are at increased gastric cancer risk. For mutation carriers, accurate initial selection for high gastric cancer risk based for instance on mutation status needs to be confirmed. Secondly, histopathological pathways of gastric carcinogenesis and the prevalence of pre-malignant gastric lesions in these carriers need to be further elucidated. Thirdly, appropriate endoscopic surveillance strategies, in terms of frequency and biopsy sampling and protocols are essential. Since we demonstrated a substantial gastric cancer risk in Lynch syndrome mutation carriers and further investigation is awaited, we advice a upper gastrointestinal endoscopic surveillance for MLH1 and MSH2 mutation carriers starting at 45 years, of age regardless of family history.

## REFERENCES


1 Forman D, Graham DY. Review article: impact of Helicobacter pylori on society-role for a strategy of 'search and eradicate'. *Aliment Pharmacol Ther* 2004;19 Suppl 1:17-21.

2 Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of Helicobacter pylori infection. *Clin Microbiol Rev* 2006;19:449-90.

3 Farinha P, Gascoyne RD. Helicobacter pylori and MALT lymphoma. *Gastroenterology* 2005;128: 1579-605.

4 Ferlay J BF, Pisani P, *et al.* . *GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide. IARC CancerBase No 5 version 2.0*. Lyon: IARCPress. 2004.

5 Bowles MJ, Benjamin IS. ABC of the upper gastrointestinal tract: Cancer of the stomach and pancreas. *Bmj* 2001;323:1413-6.

6 Malaty HM, Engstrand L, Pedersen NL, *et al.* Helicobacter pylori infection: genetic and environmental influences. A study of twins. *Ann Intern Med* 1994;120:982-6.

7 Doglioni C, Wotherspoon AC, Moschini A, *et al.* High incidence of primary gastric lymphoma in northeastern Italy. *Lancet* 1992;339:834-5.

8 Loffeld RJ, van der Putten AB. Changes in prevalence of Helicobacter pylori infection in two groups of patients undergoing endoscopy and living in the same region in the Netherlands. *Scand J Gastroenterol* 2003;38:938-41.

9 Ullrich A, Fischbach W, Blettner M. Incidence of gastric B-cell lymphomas: a population-based study in Germany. *Ann Oncol* 2002;13:1120-7.

10 Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, *et al.* Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. *Lancet* 1991;338:1175-6.

11 Wotherspoon AC, Doglioni C, Diss TC, *et al.* Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of Helicobacter pylori. *Lancet* 1993;342:575-7.

12 Stolte M, Bayerdorffer E, Morgner A, *et al.* Helicobacter and gastric MALT lymphoma. *Gut* 2002;50 Suppl 3:III19-24.

13 Severson RK, Davis S. Increasing incidence of primary gastric lymphoma. *Cancer* 1990;66:1283-7.

14 Gurney KA, Cartwright RA, Gilman EA. Descriptive epidemiology of gastrointestinal non-Hodgkin's lymphoma in a population-based registry. *Br J Cancer* 1999;79:1929-34.

15 Bayerdorffer E, Neubauer A, Rudolph B, *et al.* Regression of primary gastric lymphoma of mucosa-associated lymphoid tissue type after cure of Helicobacter pylori infection. MALT Lymphoma Study Group. *Lancet* 1995;345:1591-4.

16 Harris NL, Jaffe ES, Stein H, *et al.* A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994;84:1361-92.

17 Brands F, Monig SP, Raab M. Treatment and prognosis of gastric lymphoma. *Eur J Surg* 1997;163: 803-13.

18 Boot H, de Jong D. Diagnosis, treatment decisions, and follow up in primary gastric lymphoma. *Gut* 2002;51:621-2.

19 Warthin AS. Heredity with reference to carcinoma. *Arch Intern Med* 1913;9:279-96.

20 Lynch HT, Smyrk T, Lynch J. An update of HNPCC (Lynch syndrome). *Cancer Genet Cytogenet* 1997; 93:84-99.

21 Lynch HT, Krush AJ. Cancer family "G" revisited: 1895-1970. *Cancer* 1971;27:1505-11.

22 Lynch HT. Hereditary nonpolyposis colorectal cancer (HNPCC). *Cytogenet Cell Genet* 1999;86:130-5.

23 Vasen HF, den Hartog Jager FC, Menko FH, *et al.* Screening for hereditary non-polyposis colorectal cancer: a study of 22 kindreds in The Netherlands. *Am J Med* 1989;86:278-81.

24 Lynch HT, Lynch JF. Lynch syndrome: history and current status. *Dis Markers* 2004;20:181-98.

25 Wundisch T, Thiede C, Morgner A, *et al.* Long-term follow-up of gastric MALT lymphoma after Helicobacter pylori eradication. *J Clin Oncol* 2005;23:8018-24.

26 Morgner A, Miehlke S, Stolte M, *et al.* Development of early gastric cancer 4 and 5 years after complete remission of Helicobacter pylori associated gastric low grade marginal zone B cell lymphoma of MALT type. *World J Gastroenterol* 2001;7:248-53.

27 Hamaloglu E, Topaloglu S, Ozdemir A, *et al.* Synchronous and metachronous occurrence of gastric adenocarcinoma and gastric lymphoma: A review of the literature. *World J Gastroenterol* 2006;12: 3564-74.

28 Goteri G, Ranaldi R, Rezai B, *et al.* Synchronous mucosa-associated lymphoid tissue lymphoma and adenocarcinoma of the stomach. *Am J Surg Pathol* 1997;21:505-9.

29 Au WY, Gascoyne RD, Le N, *et al.* Incidence of second neoplasms in patients with MALT lymphoma: no increase in risk above the background population. *Ann Oncol* 1999;10:317-21.

30 Fischbach W, Goebeler ME, Ruskone-Fourmestraux A, *et al.* Most patients with minimal histological residuals of gastric MALT lymphoma after successful eradication of Helicobacter pylori can be managed safely by a watch and wait strategy: experience from a large international series. *Gut* 2007;56:1685-7.

31 Arista-Nasr J, Jimenez-Rosas F, Uribe-Uribe N, *et al.* Pathological disorders of the gastric mucosa surrounding carcinomas and primary lymphomas. *Am J Gastroenterol* 2001;96:1746-50.

32 Driessen A, Ectors N, Creemers J, *et al.* Intestinal metaplasia in gastric malignancy: a comparison between carcinoma and lymphoma. *Eur J Gastroenterol Hepatol* 1998;10:595-600.

33 Driessen A, Ectors N, Van Cutsem E, *et al.* Different gastritis features are linked to different gastric neoplasms. *Gastroenterol Clin Biol* 1999;23:747-53.

34 Lamarque D, Levy M, Chaumette MT, *et al.* Frequent and rapid progression of atrophy and intestinal metaplasia in gastric mucosa of patients with MALT lymphoma. *Am J Gastroenterol* 2006;101: 1886-93.

35 Harris NL, Jaffe ES, Diebold J, *et al.* World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting-Airlie House, Virginia, November 1997. *J Clin Oncol* 1999;17:3835-49.

36 Wu XC, Andrews P, Chen VW, *et al.* Incidence of extranodal non-Hodgkin lymphomas among whites, blacks, and Asians/Pacific Islanders in the United States: anatomic site and histology differences. *Cancer Epidemiol* 2009;33:337-46.

37 Yoshihara M, Hiyama T, Yoshida S, *et al.* Reduction in gastric cancer mortality by screening based on serum pepsinogen concentration: a case-control study. *Scand J Gastroenterol* 2007;42:760-4.

38 Leung WK, Wu MS, Kakugawa Y, *et al.* Screening for gastric cancer in Asia: current evidence and practice. *Lancet Oncol* 2008;9:279-87.

39 Miki K, Morita M, Sasajima M, *et al.* Usefulness of gastric cancer screening using the serum pepsinogen test method. *Am J Gastroenterol* 2003;98:735-9.

40 de Vries AC, Haringsma J, Kuipers EJ. The detection, surveillance and treatment of premalignant gastric lesions related to Helicobacter pylori infection. *Helicobacter* 2007;12:1-15.

41 Watabe H, Mitsushima T, Yamaji Y, *et al.* Predicting the development of gastric cancer from combining Helicobacter pylori antibodies and serum pepsinogen status: a prospective endoscopic cohort study. *Gut* 2005;54:764-8.

42 Vaananen H, Vauhkonen M, Helske T, *et al.* Non-endoscopic diagnosis of atrophic gastritis with a blood test. Correlation between gastric histology and serum levels of gastrin-17 and pepsinogen I: a multicentre study. *Eur J Gastroenterol Hepatol* 2003;15:885-91.

43 Storskrubb T, Aro P, Ronkainen J, *et al.* Serum biomarkers provide an accurate method for diagnosis of atrophic gastritis in a general population: The Kalixanda study. *Scand J Gastroenterol* 2008: 1-8.

44 Kuipers EJ. In through the out door: serology for atrophic gastritis. *Eur J Gastroenterol Hepatol* 2003;15:877-9.

45 Ley C, Mohar A, Guarner J, *et al.* Screening markers for chronic atrophic gastritis in Chiapas, Mexico. *Cancer Epidemiol Biomarkers Prev* 2001;10:107-12.

46 Bado A, Levasseur S, Attoub S, *et al.* The stomach is a source of leptin. *Nature* 1998;394:790-3.

47 Francois F, Roper J, Goodman AJ, *et al.* The association of gastric leptin with oesophageal inflammation and metaplasia. *Gut* 2008;57:16-24.

48 de Vries AC, van Grieken NC, Looman CW, *et al.* Gastric cancer risk in patients with premalignant gastric lesions: a nationwide cohort study in the Netherlands. *Gastroenterology* 2008;134:945-52.

49 de Vries AC, Haringsma J, de Vries RA, *et al.* The yield of endoscopic surveillance of pre-malignant gastric lesions: optimalization of biopsy strategies. *submitted* 2009.

50 de Vries AC, Meijer GA, Looman CW, *et al.* Epidemiological trends of pre-malignant gastric lesions: a long-term nationwide study in the Netherlands. *Gut* 2007;56:1665-70.

51 Ortner MA, Ebert B, Hein E, *et al.* Time gated fluorescence spectroscopy in Barrett's oesophagus. *Gut* 2003;52:28-33.

52 Mayinger B, Jordan M, Horbach T, *et al.* Evaluation of in vivo endoscopic autofluorescence spectroscopy in gastric cancer. *Gastrointest Endosc* 2004;59:191-8.

53 Kato M, Kaise M, Yonezawa J, *et al.* Autofluorescence endoscopy versus conventional white light endoscopy for the detection of superficial gastric neoplasia: a prospective comparative study. *Endoscopy* 2007;39:937-41.

54 Dunbar K, Canto M. Confocal endomicroscopy. *Curr Opin Gastroenterol* 2008;24:631-7.

55 Mouzyka S, Fedoseeva A. Chromoendoscopy with hematoxylin in the classification of gastric lesions. *Gastric Cancer* 2008;11:15-21; discussion -2.

56 Shaw D, Blair V, Framp A, *et al.* Chromoendoscopic surveillance in hereditary diffuse gastric cancer: an alternative to prophylactic gastrectomy? *Gut* 2005;54:461-8.

57 Uedo N, Ishihara R, Iishi H, *et al.* A new method of diagnosing gastric intestinal metaplasia: narrow-band imaging with magnifying endoscopy. *Endoscopy* 2006;38:819-24.

58 Nakayoshi T, Tajiri H, Matsuda K, *et al.* Magnifying endoscopy combined with narrow band imaging system for early gastric cancer: correlation of vascular pattern with histopathology (including video). *Endoscopy* 2004;36:1080-4.

59 East JE, Tan EK, Bergman JJ, *et al.* Meta-analysis: narrow band imaging for lesion characterization in the colon, oesophagus, duodenal ampulla and lung. *Aliment Pharmacol Ther* 2008;28:854-67.

60 Rugge M, Genta RM. Staging gastritis: an international proposal. *Gastroenterology* 2005;129:1807-8.

61 Rugge M, Correa P, Di Mario F, *et al.* OLGA staging for gastritis: a tutorial. *Dig Liver Dis* 2008;40: 650-8.

62 Rugge M, Meggio A, Pennelli G, *et al.* Gastritis staging in clinical practice: the OLGA staging system. *Gut* 2007;56:631-6.

63 Satoh K, Osawa H, Yoshizawa M, *et al.* Assessment of atrophic gastritis using the OLGA system. *Helicobacter* 2008;13:225-9.

64 Guarner J, Herrera-Goepfert R, Mohar A, *et al.* Interobserver variability in application of the revised Sydney classification for gastritis. *Hum Pathol* 1999;30:1431-4.
65 Gylling A, Abdel-Rahman WM, Juhola M, *et al.* Is gastric cancer part of the tumour spectrum of hereditary non-polyposis colorectal cancer? A molecular genetic study. *Gut* 2007;56:926-33.
66 Watson P, Vasen HF, Mecklin JP, *et al.* The risk of extra-colonic, extra-endometrial cancer in the Lynch syndrome. *Int J Cancer* 2008;123:444-9.
67 Park YJ, Shin KH, Park JG. Risk of gastric cancer in hereditary nonpolyposis colorectal cancer in Korea. *Clin Cancer Res* 2000;6:2994-8.
68 Aarnio M, Sankila R, Pukkala E, *et al.* Cancer risk in mutation carriers of DNA-mismatch-repair genes. *Int J Cancer* 1999;81:214-8.
69 Goecke T, Schulmann K, Engel C, *et al.* Genotype-phenotype comparison of German MLH1 and MSH2 mutation carriers clinically affected with Lynch syndrome: a report by the German HNPCC Consortium. *J Clin Oncol* 2006;24:4285-92.
70 Vasen HF, Stormorken A, Menko FH, *et al.* MSH2 mutation carriers are at higher risk of cancer than MLH1 mutation carriers: a study of hereditary nonpolyposis colorectal cancer families. *J Clin Oncol* 2001;19:4074-80.
71 Kastrinos F, Stoffel EM, Balmana J, *et al.* Phenotype comparison of MLH1 and MSH2 mutation carriers in a cohort of 1,914 individuals undergoing clinical genetic testing in the United States. *Cancer Epidemiol Biomarkers Prev* 2008;17:2044-51.
72 Wijnen J, de Leeuw W, Vasen H, *et al.* Familial endometrial cancer in female carriers of MSH6 germline mutations. *Nat Genet* 1999;23:142-4.
73 Wu Y, Berends MJ, Mensink RG, *et al.* Association of hereditary nonpolyposis colorectal cancer-related tumors displaying low microsatellite instability with MSH6 germline mutations. *Am J Hum Genet* 1999;65:1291-8.
74 ten Kate GL, Kleibeuker JH, Nagengast FM, *et al.* Is surveillance of the small bowel indicated for Lynch syndrome families? *Gut* 2007;56:1198-201.
75 Koornstra JJ, Mourits MJ, Sijmons RH, *et al.* Management of extracolonic tumours in patients with Lynch syndrome. *Lancet Oncol* 2009;10:400-8.
76 Vasen HF, Moslein G, Alonso A, *et al.* Guidelines for the clinical management of Lynch syndrome (hereditary non-polyposis cancer). *J Med Genet* 2007;44:353-62.
77 Hendriks YM, de Jong AE, Morreau H, *et al.* Diagnostic approach and management of Lynch syndrome (hereditary nonpolyposis colorectal carcinoma): a guide for clinicians. *CA Cancer J Clin* 2006;56:213-25.
78 Lindor NM, Petersen GM, Hadley DW, *et al.* Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: a systematic review. *Jama* 2006;296:1507-17.
79 Ye W, Held M, Lagergren J, *et al.* Helicobacter pylori infection and gastric atrophy: risk of adenocarcinoma and squamous-cell carcinoma of the esophagus and adenocarcinoma of the gastric cardia. *J Natl Cancer Inst* 2004;96:388-96.
80 Iijima K, Koike T, Abe Y, *et al.* Extensive gastric atrophy: an increased risk factor for superficial esophageal squamous cell carcinoma in Japan. *Am J Gastroenterol* 2007;102:1603-9.

# Summary

## SUMMARY

*Helicobacter pylori* infection affects at least 50% of the world population. The chronic inflammation caused by *H. pylori* can progress to pre-malignant gastric lesions, gastric adenocarcinoma and gastric MALT lymphoma. The widespread high prevalence of *H. pylori* explains that gastric cancer remains the fourth most common cancer and second leading cause of cancer related death worldwide. For these reasons, data on epidemiology and screening and surveillance options for gastric cancer in patients with *H. pylori*-associated malignancies may lead to a reduction in gastric cancer mortality.

In the first chapter the aims and outline of this these are described.

In the second chapter of this thesis we describe a pilot study on the association between *H. pylori* susceptibility and genetic factors. Overall, 277 *H. pylori*-positive patients and 728 *H. pylori*-negative patients were included in this study. Three single nucleotide polymorphisms (SNPs) in two loci demonstrated a significant association with *H. pylori* infection ($p \leq 0.05$). All three SNPs resided in unannotated regions, two on chromosome 2 (rs17015126 and rs1816653) and one on chromosome 11 (rs1939842). For these SNPs combined p-values and Odds ratios were calculated for the total cohort. All showed suggestive genome-wide associations with *H. pylori* with an OR of 2.5 (95% CI 1.7-3.5; p = 2.8 x 10-7) for rs17015126, an OR of 2.7 (95% CI 1.7-4.4; p = 2.6 x 10-5) for rs1816653 and an OR of 0.6 (95% CI 0.5-0.8; p = 2.9 x 10-5) for rs1939842. Unfortunately, the precise identity of these SNPs and the function of the nearest genes remain unknown. Therefore, future (basic) research is necessary to confirm this association and to provide insights in identity of the underlying loci.

In the third chapter we evaluated the epidemiology of gastric MALT lymphoma, and the gastric adenocarcinoma risk of patients with gastric MALT lymphoma. Firstly, we demonstrated that the incidence of gastric MALT lymphoma increased from 1991-1997. However thereafter, a rapid decline in gastric MALT lymphoma incidence was observed. This decline is in part explained by the declining *H. pylori* prevalence in Western countries. Furthermore, we showed that patients with gastric MALT lymphoma had a 6-fold increased risk for gastric cancer in comparison with the general population ($p<0.001$). In 90% of patients with diagnosis of gastric cancer, gastric cancer was diagnosed simultaneously or after gastric MALT lymphoma diagnosis, with a median interval of 6.0 years (range1-7). We concluded that accurate endoscopic and histologic re-evaluation after diagnosis for gastric MALT lymphoma is highly warranted.

In addition, the prevalence and severity of pre-malignant gastric lesions in patients with gastric MALT lymphoma may indicate an increased gastric cancer risk. Therefore in chapter four we evaluated the differences between the prevalence of pre-malignant gastric lesions

in gastric MALT lymphoma patients with a subsequent diagnosis of gastric cancer and those without. No differences were demonstrated in the prevalence and severity of pre-malignant gastric lesions of gastric MALT lymphoma patients with a subsequent diagnosis of gastric cancer or those without. However, surprisingly, advanced pre-malignant gastric lesions were common in both patients with subsequent gastric cancer and patients without gastric cancer development. This indicated that endoscopic and histopathologic surveillance with specific attention to the severity of pre-malignant gastric lesions after diagnosis of gastric MALT lymphoma is highly warranted.

In the fifth till seventh chapter we evaluated screening and surveillance options for patients with pre-malignant gastric lesions. In the fifth chapter we described a large cohort of 119 patients with a previous diagnosis of intestinal metaplasia or dysplasia and 98 patients with no diagnosis of advanced precursor lesions. We demonstrated that serum leptin levels can serve as extra tool to predict patients with high gastric cancer risk in combination with the established risk factors, in particular male sex, advancing age, and low serum pepsinogen I levels. However, our results showed that the additional value of this non-invasive marker is rather low. In the sixth chapter, we presented a cohort of 43 patients with a previous diagnosis of intestinal metaplasia and dysplasia. These patients underwent surveillance endoscopy with conventional white light and narrow band imaging (NBI). We showed that the sensitivity for the detection of advanced pre-malignant gastric lesions was 71% for NBI and 51% for the conventional white light endoscopy. We concluded therefore that NBI considerably increases the diagnostic yield of the detection of gastric intestinal metaplasia and dysplasia, compared to routine WLE. NBI therefore seems superior to WLE in the surveillance of patients with advanced gastric precursor lesions.

We proposed in the seventh chapter a new histological staging system for estimating gastric cancer risk in patients with pre-malignant gastric lesions. This new staging system was based on the grading of intestinal metaplasia (OLGIM) instead of the recently proposed OLGA staging system which is based on the grading of atrophic gastritis. We showed that the interobserver agreement was substantial for atrophic gastritis (kappa value=0.6) and almost perfect for intestinal metaplasia (kappa value=0.9). In addition, the interobserver agreement was improved for all stages of the OLGIM compared to the OLGA. Moreover, the correlation with the severity of gastritis remained at least as strong. Therefore, we concluded that the OLGIM may be preferred over the OLGA for the prediction of gastric cancer risk in patients with pre-malignant gastric lesions.

In addition to the increased gastric cancer risk in patients with gastric MALT lymphoma and pre-malignant gastric lesions, Lynch syndrome mutation carriers seem to have an increased gastric cancer risk, too. In chapter 8 of this thesis we described the incidence trends of gastric cancer and the gastric cancer risk in a cohort of 2014 mutation carriers. In total, 32 (1.6%)

Lynch syndrome mutation carriers were diagnosed with gastric cancer. Firstly, we showed that the standardized incidence rate of gastric cancer in Lynch syndrome mutation carriers decreased from 4.0 (95% CI 1.5-8.6) in the years 1970-1979 to 2.1 (95% CI 0.6-5.3) in 1990-1999 (p=0.03). Secondly, we demonstrated a lifetime risk of developing gastric cancer of 8.0% for males and 5.3% for females (p=0.02). This risk was particularly increased in patients with MLH1 and MSH2 mutations. For these reasons, we concluded that the incidence of gastric cancer showed a non-significant decrease during the past decades and that surveillance upper GI-endoscopy for Lynch syndrome patients carrying an MLH1 or MSH2 mutation should be considered, due to the substantial gastric cancer risk in these patients.

In chapter 9 of this thesis, we evaluated the risk of esophageal squamous cell carcinoma in patients with gastric atrophy, since an explanation for this association remains largely unclear. Of the 97728 patients that were included with a first diagnosis of gastric atrophy, 126 patients developed ESCC. An overall relative risk of 2.2 was demonstrated for the development of ESCC in gastric atrophy patients. However, the risk of ESCC did not increase with the severity of gastritis, with relative risks of 2.1 for development of ESCC in patients with intestinal metaplasia and 2.3 for patients with dysplasia. Moreover, a similar association was demonstrated between gastric atrophy and small cell lung carcinoma. Hence, a causal relationship between gastric atrophy and the development of ESCC seems unlikely.

In the remaining chapters the main findings of this thesis are discussed and future directions for further research on *H. pylori*-associated malignancies are provided.

# Samenvatting

## SAMENVATTING

Ongeveer 50% van de wereldbevolking heeft een *Helicobacter pylori* infectie doorgemaakt. De chronische ontsteking die wordt veroorzaakt door *Helicobacter pylori* kan leiden tot pre-maligne maagafwijkingen, maagkanker en een MALT lymfoom van de maag. De wijd verspreide prevalentie van *H. pylori* is een verklaring voor het feit dat maagkanker de vierde meest voorkomende kanker is en de tweede aan kanker gerelateerde doodsoorzaak wereldwijd. Kennis van de epidemiologie en screening en surveillance opties voor maagkanker in patiënten met *Helicobacter pylori* - geassocieerde maligniteiten kunnen leiden tot een reductie in maagkanker mortaliteit.

In het eerste hoofdstuk worden de doelen en de achtergrond van dit proefschrift beschreven.

In het tweede hoofdstuk van dit proefschrift beschrijven wij in een pilot studie de associatie tussen *H. pylori* ontvankelijkheid en genetische factoren. In totaal werden er 277 *H. pylori*-positieve patiënten geincludeerd en 728 *H. pylori*-negatieve patiënten. Drie SNP's op twee loci toonden een significante associatie met *H. pylori* infectie ($p<0.05$). Deze drie SNPs lagen alle drie in nog onbeschreven regionen van het DNA; twee op chromosoom 2 (rs17015126 and rs1816653) en één op chromosoom 11 (rs1939842). Gecombineerde p-waardes en Odds ratios zijn berekend voor deze drie SNPs voor het hele cohort. Alle SNPs toonden suggestieve genoom brede associates met *H. pylori* met een OR van 2.5 (95% CI 1.7-3.5; $p = 2.8 \times 10\text{-}7$) voor rs17015126, een OR van 2.7 (95% CI 1.7-4.4; $p = 2.6 \times 10\text{-}5$) voor rs1816653 en een OR van 0.6 (95% CI 0.5-0.8; $p = 2.9 \times 10\text{-}5$) voor rs1939842. Helaas is de precieze identiteit van de SNPs, evenals de functie van de dichtstbij gelegen genen nog onbekend. Om deze reden is toekomstig (basaal) onderzoek noodzakelijk om de gevonden associaties te bevestigen en nieuw inzichten te bieden in de identiteit van de onderliggende loci.

In het derde hoofdstuk wordt de epidemiologie van MALT lymfomen van de maag en het maagkanker risico van patiënten met een MALT lymfoom van de maag geevalueerd. Ten eerste toonden we een toename van de incidentie van MALT lymfomen van de maag in de periode 1991-1997, echter daarna werd een snelle afname in incidentie waargenomen. Deze afname wordt gedeeltelijk verklaard door de afname in *H. pylori* prevalentie in Westerse landen. Daarnaast demonstreerden we dat patiënten met een MALT lymfoom van de maag een 6 keer hoger risico hebben op het ontwikkelen van maagkanker in vergelijking met de Nederlandse populatie ($p<0.001$). In 90% van de patiënten met maagkanker werd maagkanker gelijktijdig of na de diagnose MALT lymfoom van de maag gesteld, met een mediaan interval van 6 jaar (range 1-7 jaren). Om deze redenen concludeerden we dat accurate endoscopische en histologische herevaluatie na een diagnose MALT lymfoom van de maag strict noodzakelijk is.

Bovendien kan de prevalentie en ernst van pre-maligne maagafwijkingen in patiënten met een MALT lymfoom van de maag wijzen op een verhoogd maagkanker risicio. In hoofdstuk vier evalueerden we daarom de verschillen tussen de prevalentie van pre-maligne maagafwijkingen in patiënten met een MALT lymfoom van de maag en daaropvolgend een diagnose maagkanker en patiënten met een MALT lymfoom van de maag zonder maagkanker ontwikkeling. Ook de prevalentie van pre-maligne maagafwijkingen in patiënten met een diffuus grootcellig B-cel lymfoom die later maagkanker ontwikkelden werd geevalueerd. Er werden geen verschillen gevonden in de prevalentie en ernst van pre-maligne maagafwijkingen tussen patiënten met maagkanker en zonder maagkanker of patiënten met een MALT lymfoom van de maag en een diffuus grootcellig B-cel lymfoom. Het is echter wel verrassend dat gevorderde pre-maligne maagafwijkingen veelal gezien worden in zowel patiënten met en zonder een uiteindelijke diagnose van maagkanker. Dit toont opnieuw dat endoscopische en histologische herevaluatie van groot belang is na een diagnose MALT lymfoom of diffuus grootcellig B-cel lymfoom.

In het vijfde tot zevende hoofdstuk beschrijven wij screening en surveillance mogelijkheden voor patiënten met pre-maligne maagafwijkingen. In het vijfde hoofdstuk beschrijven we een groot cohort van 119 patiënten met een eerdere diagnose van intestinale metaplasie of dysplasie en 98 patiënten zonder deze pre-maligne maagafwijkingen. We toonden dat serum leptine niveaus in combinatie met de bekende risicofactoren, namelijk mannelijk geslacht, oudere leeftijd, en lage serum pepsinogeen I niveaus, kunnen dienen als extra marker om patiënten met een hoog maagkanker risico te voorspellen. Echter onze resultaten toonden dat de toegevoegde waarde van deze non-invasieve marker gering is. In het zesde hoofdstuk presenteerden we een cohort van 43 patiënten met een eerdere diagnose van intestinale metaplasie of dysplasie die surveillance endoscopie met het conventionele wit licht en met Narrow band imaging (NBI) ondergingen. We toonden dat de sensitiviteit voor de detectie van gevorderde pre-maligne maagafwijkingen 71% was voor NBI en 51% voor de conventionele wit licht endoscopie. Om deze reden concludeerden we dat NBI de diagnostische opbrengst voor de detectie van intestinale metaplasie en dysplasie significant verhoogd. NBI lijkt daarom superieur boven wit licht endoscopie voor de surveillance van patiënten met gevorderde pre-maligne maagafwijkingen.

In het zevende hoofdstuk stellen wij een nieuw histologisch stadiërings systeem voor om het maagkanker risico in patiënten met pre-maligne maagafwijkingen te schatten. Dit nieuwe stadiërings systeem is gebaseerd op het graderen van intestinale metaplasia (OLGIM) in plaats van het recent voorgestelde OLGA stadiërings systeem wat gebaseerd is op het graderen van atrofische gastritis. We toonden aan dat de interobserver overeenstemming substantieel was voor een diagnose atrofische gastritis (kappa=0.6), maar bijna perfect was voor een diagnose intestinale metaplasie (kappa =0.9). Daarnaast was de interobserver overeenstemming in vergelijking met de OLGA stadia verbeterd voor alle OLGIM stadia.

Bovendien was de correlatie met de ernst van de gastritis tenminste even goed. Om deze redenen concludeerden we dat de OLGIM verkozen dient te worden boven de OLGA om het maagkanker risico te schatten in patiënten met pre-maligne maagafwijkingen.

Naast het verhoogde maagkanker risico in patiënten met een MALT lymfoom van de maag en pre-maligne maagafwijkingen, lijken Lynch syndroom mutatie dragers ook een verhoogd maagkanker risicio te hebben. In hoofdstuk 8 van dit proefschrift beschrijven we de incidentie van maagkanker en het maagkanker risico in een cohort van 2014 mutatie dragers. In totaal werd in 32 (1.6%) van de Lynch syndroom mutatie dragers maagkanker gediagnosticeerd. Ten eerste toonden we dat de gestandaardiseerde incidentie ratios van maagkanker in Lynch syndroom mutatie dragers afnam van 4.0 (95% CI 1.5-8.6) in de periodee 1970-1979 naar 2.1 (95% CI 0.6-5.3) in 1990-1999 (p=0.03). Ten tweede demonstreerden we een risico op het ontwikkelen van kanker gedurende een leven van 8.0% voor mannen en 5.3% voor vrouwen (p=0.02). Dit risico was vooral verhoogd in patiënten met een MLH1 of MSH2 mutatie. Om deze reden concludeerden we dat de incidentie van maagkanker een niet significante verlaging toonde in de afgelopen decaden en dat surveillance endoscopie voor Lynch syndroom patiënten met een MLH1 of MSH2 mutatie overwogen moet worden vanwege het substantiele maagkanker risico in deze patiënten.

We evalueerden het risico op plaveiselcel carcinomen van de slokdarm in patiënten met atrofische gastritis in hoofdstuk 9 van dit proefschrift, omdat een verklaring voor de eerder beschreven associatie hiertussen onduidelijk is. In het studie cohort dat bestond uit 97 728 patiënten met een eerste diagnose van atrofische gastritis, ontwikkelden 126 patiënten een plaveiselcel carcinoom van de slokdarm. Een totaal relatief risico van 2.2 was berekend voor het ontwikkelen van plaveiselcel carcinomen van de slokdarm in patiënten met atrofische gastritis. Echter het risico op plaveiselcel carcinomen van de slokdarm was niet evenredig verhoogd met de ernst van de gastritis, we toonden namelijk een relatief risico van 2.1 voor de ontwikkeling van deze carcinomen in patiënten met intestinale metaplasie en een relatief risico van 2.3 voor patiënten met dysplasie. Bovendien werd er een zelfde associatie aangetoond tussen atrofische gastritis en kleincelling longkanker. Om deze reden lijkt een causaal verband tussen atrofische gastritis en het ontwikkelen van plaveiselcel carcinomen van de slokdarm onwaarschijnlijk.

In de laatste hoofdstukken van dit proefschrift worden de belangrijkste bevindingen bediscussieerd en toekomstige richtingen voor verder onderzoek naar *H. pylori*-geassocieerde maligniteiten beschreven.

# Dankwoord

## DANKWOORD

Een proefschrift voltooïen kan niet zonder de hulp van anderen, graag wil ik iedereen die bijgedragen heeft hartelijk bedanken. In het bijzonder gaat mijn dank uit naar:

Mijn promotor professor dr. E. J. Kuipers, beste Ernst, graag wil ik je bedanken voor het vertrouwen, je enthousiasme, je uitleg (vaak met een tekeningetje erbij waardoor iets wat ik als 'complex' beschouwde opeens buitengewoon simpel werd), de mogelijkheden die je me geboden hebt, en het opbouwend commentaar bij manuscripten dat vaak in de avonden en in alle vroegte in het weekend werd toegestuurd. Je aanstekelijke humor leidde soms tot gedenkwaardige situaties. Een goed voorbeeld: Eénmaal trof ik jou aan het einde van de dag samen met Jelle schaterlachend aan, jij had een sinaasappel voor Jelle bewaard, en Jelle een Mars voor jou. Zo vullen jullie elkaar goed aan! Ik wil je graag heel hartelijk danken voor de plezierige samenwerking. Jelle Haringsma, ook jou ben ik veel dank verschuldigd. Beste Jelle, jij leerde me dat promoveren leuk en interessant is, maar dat het nog veel belangrijker is een goede dokter te worden. Dank voor alle tijd en moeite die je in de scopieën en studies hebt gestoken.

Prof. dr. H. F. A. Vasen, beste Hans, veel dank voor de gelegenheid die je me geboden hebt om binnen de Stichting Opsporing Erfelijke Tumoren in Leiden onderzoek te doen naar maagkanker. De volgende manuscripten uit deze samenwerking, die ik altijd als heel prettig heb ervaren, zullen nu toch echt snel volgen.

Prof. dr. A. G. Uitterlinden, beste André, door jouw enthousiasme werd ik de SNP-wereld binnen getrokken, hartelijk dank voor alle steun in het mega-project. Daarnaast ook veel dank aan Lisette Stolk en Michael Verbiest, voor het inzichtelijk maken van de SNP materie en analyses.

Graag wil ik ook Nicole van Grieken bedanken. Jouw enthousiasme voor de pathologie heeft mij laten inzien dat patholoog een heel boeiend en leuk vak is. Dank voor alles wat je me geleerd hebt onder de microscoop, de ideeën voor lopend en nieuw onderzoek, maar ook de kopjes koffie en gezelligheid tijdens de bezoeken aan het VUMC.

Ook de overige leden van de grote en kleine commissie wil ik bedanken voor de bereidheid plaats te nemen in de commissie en de aanwezigheid op 3 september 2010.

Beste Richard de Vries en Frank ter Borg, zonder jullie uitgebreide inclusie van patiënten waren mijn artikelen niets waard geweest, dank voor jullie inzet. Daarnaast Herman van Dekken, Jos Meijer en Katharina Biermann, dank voor de deskundigheid en het groot aantal coupes dat jullie voor mij bekeken en gescoord hebben. Tevens gaat mijn dank uit naar Marielle Casparie voor alle ondersteuning bij de PALGA projecten.

Statistici Ewout Steyerberg en Caspar Looman, graag wil ik jullie bedanken voor jullie geduld, de kunst waarover jullie beiden beschikken om lastige problemen om te toveren tot begrijpelijke stof en de input bij de verschillende artikelen en revisies.

Eveneens gaat mijn dank uit naar alle endoscopie-verpleegkundige en secretaresses van het EMC maar ook van de Stichting Opsporing Erfelijke Tumoren voor de ondersteuning en hulp bij de inclusie van patiënten. Wendy Holleman, veel dank dat ik altijd met mijn vragen bij je terecht kon.

Een promotie onderzoek loopt meestal niet zoals gepland, zo ook bij mij. Eigenlijk zou ik starten met een ratten- en muizenmodel, maar het liep anders. Lieve Annemarie de Vries daarvoor wil ik je graag bedanken. Zonder het aanbod om jouw 'maagmaatje' te worden was dit een heel ander proefschrift geworden. Dank voor al je steun, je altijd goede tips en adviezen, en de leuke tijd die we samen hebben gehad in zowel het Erasmus als in het SFG. Hopelijk gaan we in de komende jaren nog veel meer samenwerken.

Caroline den Hoed, fijn dat jij kwam om de maag-projecten voort te zetten. Lieve Caroline, jouw tomeloze inzet niet alleen in je werk maar ook daarbuiten zorgen ervoor dat alles wat je wilt je ook lukt. Daar heb ik groot respect voor. Dank voor alle ondersteuning op het werkgebied, de etentjes en de adviezen bij het zoeken naar woonruimte.

Dan natuurlijk wil ik het MDL-lab hartelijk bedanken voor de leuke tweeëneenhalf jaar die ik bij jullie heb doorgebracht. De stafleden, Hans Kusters, Hanneke van Vuuren, André Boonstra, Andrea Woltman, Luc van der Laan, Jaap Kwekkeboom en Ron Smits, dank voor jullie laagdrempeligheid en het feit dat ik bij alle labtechnische problemen bij jullie mocht aankloppen. De diagnostiek, Angela Heijens, Martine Ouwendijk, Nicole Nagtzaam en Jan Francke dank voor jullie vrolijkheid en hulp bij alle ELISA's en andere proeven. Verder wil ik Anouk (zonder jou had ik er niets van begrepen!), Chantal, Katinka, Lianne, Jeroen, Hans, Mark, Marolein O, Pieter-Jan, Werner, Wendy, Scot, Shanta, Suomi, en Thanya bedanken voor de gezellige tijd. Patrick, Anthonie, en Antoine dank voor jullie gezelligheid, grapjes (en outfits) bij de vele borrels en feestjes. Niet te vergeten, Anthonie ook bedankt voor het organiseren van het kampeerweekend! Mijn (oud)-kamergenoten, Jeroen, Clara, Abdullah, Celine wil ik bedanken voor de gezellige tijd. Jasper (the hottest person in the room?!) dank voor je enthousiasme ook wanneer ik je weer eens vroeg iets basaals uit te leggen. Dear Viv and Lally, thanks for all the nice chats mostly on non-medical or science stuff and the cheerful welcome you gave me every time I returned to the lab. Marjolein Sikkema, lieve Marjolein, als maatje ongeveer dezelfde tijd op het lab, dank voor alle gezelligheid, lief en leed delen gaat blijkbaar erg goed als je met de rug naar elkaar toezit, veel plezier en succes in Utrecht.

Voorts wil ik alle arts-onderzoekers Aefke, Aria, Desiree, Dew, Edith, Eva, Femme, Jerome, Jildou, Jur, Lieke, Margot, Nicoline, Paul, Robert, Vera en Vincent bedanken voor de gezellige tijd die ik heb gehad, soms tijdens de lunch, maar vooral tijdens de borrels, feestjes en niet te vergeten alle congressen.

Daarnaast de stafleden en collega's uit het Sint Franciscus Gasthuis, dank voor de prettige samenwerking, maar ook voor alle tips en adviezen omtrent deze promotie.

Tijdens mijn promotie-onderzoek heb ik veel gepiekerd over allerlei zinnige en onzinnige zaken, maar uiteindelijk zijn er toch maar een paar dingen echt belangrijk en dat zijn vrienden en familie.

Lieve Daphne, Monique en Aukje, dank voor de gezellige jaren in Utrecht zo zie je maar weer waar zo'n introductieweek goed voor is. Fijn om ervaringen met jullie te kunnen uitwisselen maar ook om te kletsen over niet medische onderwerpen. Lieve Miek, dank voor al je hulp bij deze promotie, je positieve instelling en je inlevingsvermogen. Hopelijk zien we elkaar nu weer wat vaker in Rotterdam, Utrecht of elders! Lieve Flavours: Aef, Freek, Fre, Jes, Ka, Lau, Lies, Mar, Mat, Mies, en Wol, ik ben blij dat jullie ook genoegen namen met mailtjes en telefoontjes voor elke keer dat ik dienst had of achter mijn computer zat als er weer eens een vrimibo/zamibo was. Jullie interesse werd er zeker niet minder om. Dank! Lieve huisgenoten, Suus, Susan en Heske, bedankt voor jullie niet aflatende steun bij dit soms wat lastige project dat pieken en dalen heeft gekend. Alle avondjes (Bartje en Weeshuis), weekendjes en vakanties hebben geleid tot een fantastische basis voor hopelijk een levenslange vriendschap (en natuurlijk een perfecte klaverjas-eenheid).

Dan wil ik graag Stefan, Cockie, Felix en Daniëlle bedanken voor alle interesse en het warme welkom in de schoonfamilie.

Mijn familie: lieve oma, dank voor al uw wijze raad en (Amsterdamse) humor. Lieve zusjes en Maarten; lieve Elissa dank voor de waardering en steun vanaf de zijlijn, ik hoop dat ik in de toekomst nu weer vaker mee kan doen aan alle spelletjes thuis. Lieve Annette en Maarten, dank voor jullie interesse de afgelopen jaren, lieve net, wat heb ik het getroffen met zo'n tweelingzus! Dank voor het ontwerpen van de kaft in je spaarzame tijd, ik ben er ontzettend blij mee.

Lieve pap, door jouw opvoeding met alle (soms exotische maar met name cultureelverantwoorde) reizen die we gemaakt hebben, heb je me geleerd dat alles relatief is en dat we vooral moeten genieten van het leven. Dank, dit kwam tijdens mijn promotie-onderzoek goed van pas wanneer ik verzandde in 'wetenschappelijke' problemen. Lieve Urdice, dankjewel voor de welkome afleiding op zijn tijd en de heerlijke diners bij jouw thuis!

Lieve mam, jouw oor heeft veel te verduren gehad als ik je weer eens belde dat ik mijn computer uit het raam wilde kieperen. Dank dat jij er altijd een positieve draai aan geeft, voor je onuitputtelijke vertrouwen, maar ook raad waar ik het niet altijd mee eens ben maar die wel vaak blijkt te kloppen! Lieve Willem, dank voor al je hulp bij de totstandkoming van dit proefschrift, ook jouw steun ervaar ik echt als onvoorwaardelijk!

Last but not least, Victor, lieve Victor, hoe kan ik je bedanken, ik weet niet waar ik moet beginnen. Ook jij hebt het de afgelopen jaren met al de rondjes Randstad zwaar te verduren gehad, maar klagen hoorde ik je nooit! Dank dat jij met je positieve instelling en humor altijd weer iets leuks bedacht (bijvoorbeeld 'zullen we weer op reis gaan?') als ik in totaal irrelevante promotie-stress verkeerde. Ik ben zo blij dat ik met jou het leven mag delen, iedere dag samen is een dag met een extra glansje.

# Curriculum vitae

## CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 18 mei 1982 in Amsterdam. Na het behalen van haar VWO examen aan het Mendel College te Haarlem in 2000 startte zij haar geneeskunde studie aan de universiteit van Utrecht. Gedurende zes jaar deed zij verschillende onderzoeken onder andere in Léon te Nicaragua. Op 22 december 2006 behaalde zij haar arts-examen. Vanaf 1 januari 2007 is zij begonnen met haar promotie onderzoek naar *Helicobacter pylori* geassocieerde maligniteiten van de maag op de afdeling Maag-, Darm-, Leverziekten van het Erasmus MC in Rotterdam, onder begeleiding van Prof. dr. E. J. Kuipers. Dit onderwerp vormt de basis van haar proefschrift. Per juni 2009 is zij gestart met de opleiding tot Maag-, Darm-, Leverarts via het Erasmus MC (opleiders: Dr. R. A. De Man en Prof. dr. E. J. Kuipers), waarbij de vooropleiding thans wordt verricht in het SFG (opleiders: Dr. A. P. Rietveld en Dr. H. C. T. Van Zaanen).

# Portfolio

## PHD PORTFOLIO

Name PhD student: Lisette Capelle
Erasmus MC department: Gastroenterology and Hepatology
PhD period: 01/01/2007 – 03/09/2010
Promoter: prof. dr. E. J. Kuipers

## INTERNATIONAL CONFERENCES: ORAL PRESENTATIONS

*NVGE 2007, Veldhoven, The Netherlands*
Considerable gastric cancer risk during first year after diagnosis of gastric MALT lymphoma

*Digestive Disease Week 2008, San Diego, USA*
High adenocarcinoma risk after diagnosis of gastric MALT lymphoma: a long-term nationwide study

*NVGE 2008, Veldhoven, The Netherlands*
Serum levels of leptin: a potential marker for patients at high risk of gastric cancer?

*UEGW 2008, Vienna, Austria*
Epidemiology of gastric MALT lymphoma: a long-term nationwide study in the Netherlands
High adenocarcinoma risk after diagnosis of gastric MALT lymphoma: a long-term nationwide study
Options of screening and surveillance of pre-malignant gastric lesions

*NVGE 2009, Veldhoven, The Netherlands*
Grading and staging gastritis with the OLGA system: intestinal metaplasia as reproducible alternative
Epidemiological time trends and gastric cancer risk in Lynch syndrome mutation carriers in the Netherlands

*Digestive Disease Week 2009, Chicago, USA*
The identification of host genetic polymorphisms for *H. pylori* infection: a Genome Wide Association study (*plenary session*)
Risk and epidemiological time trends of gastric cancer in Lynch syndrome mutation carriers

*UEGW 2009, London, Great-Britain*

Epidemiological time trends and gastric cancer risk in Lynch syndrome mutation carriers in the Netherlands

## INTERNATIONAL CONFERENCES: POSTERS

*Digestive Disease Week 2008, San Diego, USA*
Epidemiology of gastric MALT lymphoma: a long-term nationwide study (*poster of distinction*)

*Digestive Disease Week 2009, Chicago, USA*
Serum levels of leptin as marker for patients at high gastric cancer risk
Gastric cancer risk in Lynch syndrome families in the Netherlands
Epidemiological time trends of gastric cancer in Lynch syndrome in Lynch Syndrome families in the Netherlands
Grading and staging gastritis with the OLGA system: intestinal metaplasia as reproducible alternative

*UEGW 2009, London, Great-Britain*
Grading and staging gastritis with the OLGA system: intestinal metaplasia as reproducible alternative

## COURSES, SEMINARS AND WORKSHOPS

Introduction in data-analysis, August 2007
SNPs and Human Diseases, September 2008
English course, January 2008