The serological gastric biopsy in primary care : studies on atrophic gastritis

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The Serological Gastric Biopsy in Primary Care

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STUDIES ON ATROPHIC GASTRITIS

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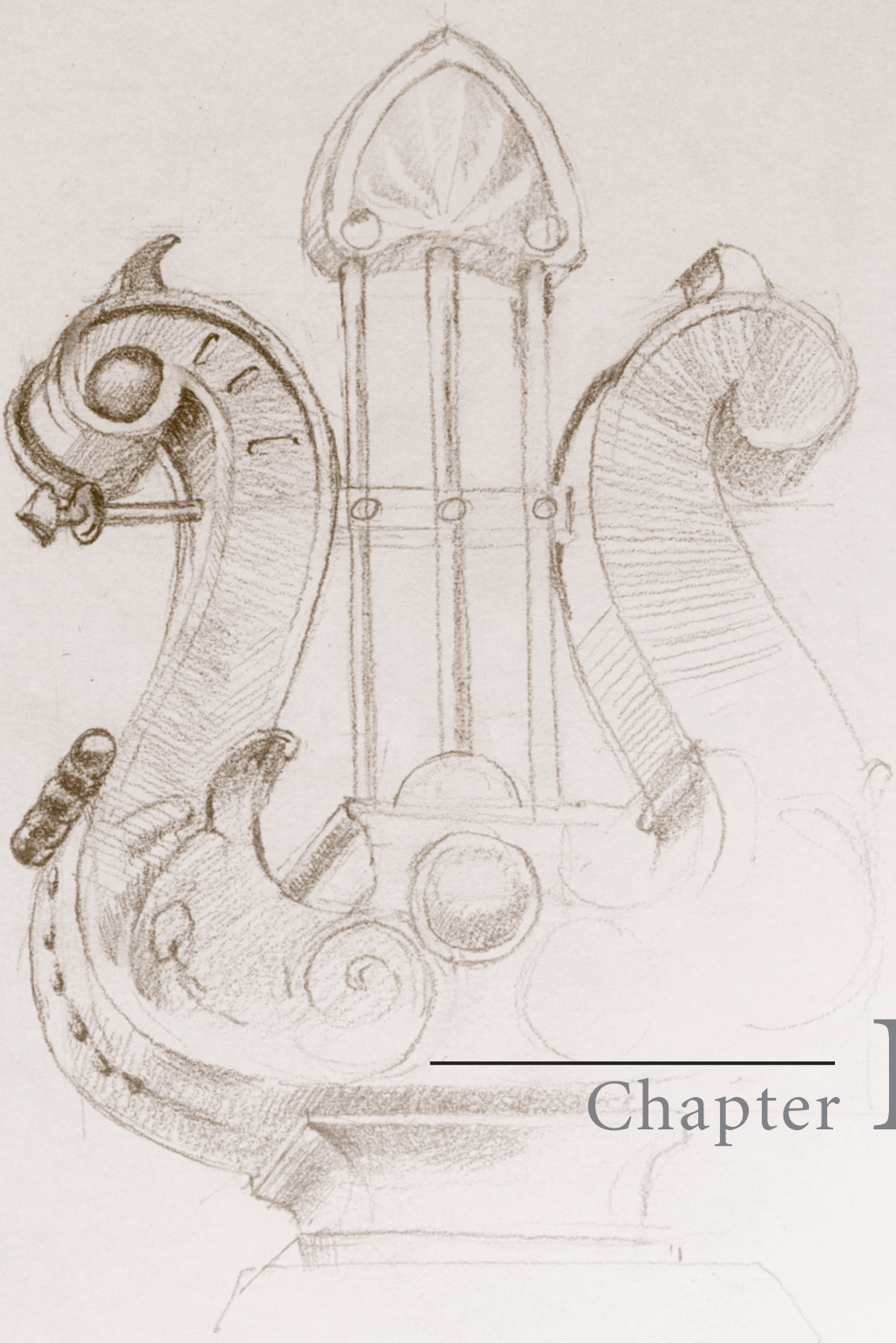

Chapter I

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The diversity is the dignity of general practice.

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Chapter I

Introduction

For centuries the human stomach was an inaccessible dark organ, hidden in the abdomen [1]. The digestive tract, including the stomach, remained an object of ill understood function.

During the last century the knowledge of stomach function and diseases was tremendously accelerated by advancing physico-optical and biochemical technology. The most accurate diagnostic method of gastric diseases is nowadays fibreoptic endoscopy with subsequent biopsy. The contribution of a non-invasive diagnostic modality for gastric disorders became apparent in the 1970s.

It has long been known that the stomach, being part of the digestive system, secretes several bio-active products with a specific cyto-secretory background. The most important biological active products are respectively, the gastric acid protease Pepsinogen, separated in two major groups: Pepsinogen (Pg) group I and group II [2,3] and the gastric acid secretagogue Gastrin [4-6]. Pg I and Pg II are produced by the glands of the fundus and corpus, Pg II also by duodenal Brunner's glands. Gastrin is produced by antral G-cells.

A healthy gastric mucosa releases normal concentrations of biomarkers in plasma. A mucosa with a hyperplastic or hypertrophic cell mass induces hypersecretion and a mucosa with an atrophic cellular structure induces hyposecretion of the biomarkers. The gastric secretory behaviour reflects the mucosal condition and cyto-secretory profiles can characterize gastric disorders, for instance hypochlorhydria is related to hypoparietalism.

Since Samloff found that serum Pg group I is produced only in the acid secretory part of the stomach [7], interest has been focused on the relation between serum

Pg I and gastric acid secretion [8]. Several studies pointed out that the concentration of Pg I in serum reflects the capacity of the gastric mucosa to secrete hydrochloric acid. The measurement of serum Pg I can be recommended as a screening test for achlorhydria [9,10]. Additionally, elevated plasma gastrin is found in a high percentage of subjects with achlorhydria [4-6].

Consequently, the serum level of the biomarkers, pepsinogen and gastrin, can be used as an indirect method providing information about the functional trophic state of the gastric mucosa. A diagnostic serum sample, a so-called "serum biopsy", offers an easy, non-invasive means to evaluate peptic secretion. It reflects the secretory condition of the stomach and predicts non-endoscopically the histological quality of the glandular layer. These qualities make the serum biopsy very attractive and appropriate to be used in the non-invasive diagnosis of gastric mucosal disorders, especially atrophic gastritis.

For a better insight in the essence of the serological gastric biopsy this introductive chapter provides a background on the normal histology and physiology of the gastric mucosa and on the clinical relevance of the serological gastric biopsy, with focus on the non-invasive diagnosis of atrophic gastritis, a well-known gastric cancer precursor lesion. Special attention is payed to the two main aetiological factors of chronic gastritis, namely *Helicobacter pylori* infection and gastric autoimmunity, whose presence are both detectable by serology.

Normal gastric mucosa

In order to understand the histo-physiological background of the serological gastric biopsy, it is essential to know what the normal gastric mucosa looks like and to understand how the normal stomach works. Therefore, the normal anatomy, histology and physiology are described.

Anatomy and histology

The stomach is situated in the abdominal cavity and exists of four anatomical zones: the cardia, the fundus, the corpus and the antrum *(fig.1)* These four zones roughly correspond to different types of mucosa. The functional unit of the gastric mucosa consists of a gastric pit (foveola), a neck region and three to five glands *(fig.2)*. The cells lining the surface epithelium are the same in all four parts of the stomach and exist of mucus producing, columnar epithelial cells. The cells of the glandular epithelium vary throughout the stomach. In the cardia, which is the most proximal part of the stomach, the glands exist of tubular, mucous secreting cells. The pit/gland ratio is about one, which means that half of the mucosal thickness is occupied by pits and the deeper half by glands.

The fundus and the corpus mucosa together are often referred to as oxyntic mucosa. Four main cell types are recognized in the oxyntic glands: mucous neck cells,

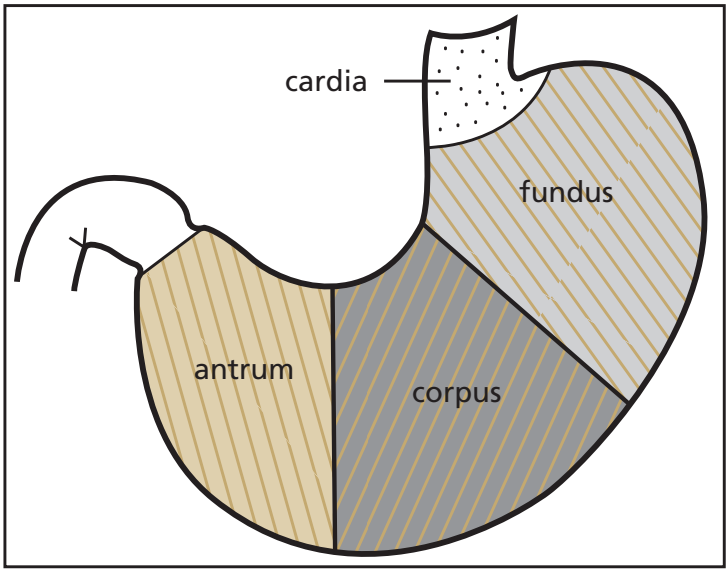

Figure 1. *Anatomical regions of the stomach: cardia, fundus, corpus and antrum*

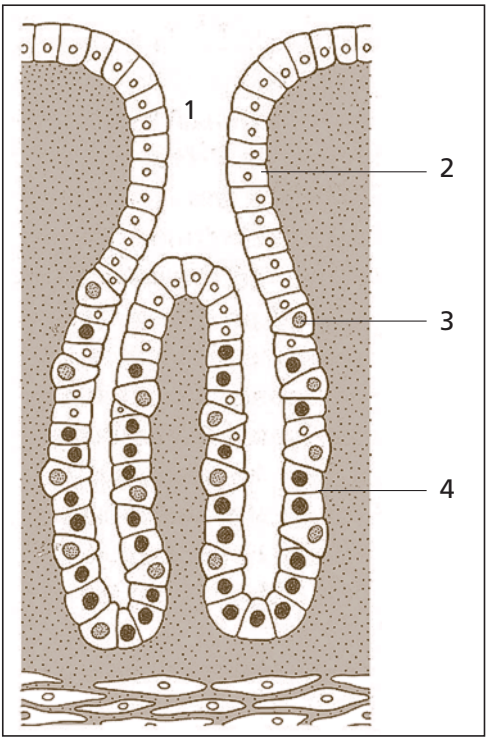

Figure 2. *Different cell types that are present in the oxyntic mucosa:*
1. gastric pit
2. columnar surface epithelium
3. parietal cell
4. chief cell

parietal or oxyntic cells, which produce HCl and intrinsic factor, chief or zymogen cells that secrete pepsinogen and lipase, and endocrine cells, which produce histamine *(fig.2)*. The pits in the oxyntic mucosa are short and comprise about 1/5 of the total thickness of the mucosa.

The antral glands contain mucin-secreting cells, resembling the mucous neck cells. Also, parietal cells are found in the antral mucosa of almost all subjects. Furthermore, gastrin-producing cells (G-cells) are detected in the glandular layer of the antrum mucosa [11].

Transitional zones form the junctions between different types of mucosa in the stomach, for example between antrum and body mucosa. In these transitional zones, one type mucosa gradually merges into the next type mucosa. The proximal border of the antrum-corpus transitional zone is defined as where the G-cells, which are not present in the corpus, disappear. The distal border of the antrum-corpus transitional zone on the other hand, lies there where the chief cells, that are absent in the antrum, disappear [12].

Physiology

The stem cells of the gastric mucosa are located in the neck region. These are relatively undifferentiated cells that proliferate, while at the same time their number remains constant.

They have the capacity to regenerate their own population and the gastric mucosal tissue after damage [13]. The descendants of the stem cells migrate either upwards towards the mucosal surface or downwards towards the glands. Upgoing cells differentiate into mucous secreting cells, while the cells that migrate towards the glandular layer differentiate into e.g. parietal cells or chief cells *(fig.3)*. It is unclear whether stem cells for the parietal cells are the same as the stem cells from which the surface epithelium originates [14]. Epithelial cells divide and migrate more rapidly in atrophic gastric mucosa than in normal mucosa [14].

After having passed through their programmed lifetime, the glandular epithelial cells physiologically undergo apoptosis, often synonymously used with the term "programmed cell death", in the deeper layer of the mucosa. As soon as the apoptotic program of a cell is completed, macrophages or adjacent epithelial cells phagocytose the remainder of the cell [15]. Apoptosis of the superficial epithelial cells takes place at the surface of the mucosa at the top of the gastric pits *(fig 3)*. It is not entirely clear whether these cells are cleared by phagoytosis or shedded into the lumen of the stomach.

The gastric superficial mucosa has a high turnover rate and the mucous-secreting cell population is replaced within 3-6 days. The parietal and chief cells in the glandular layer have a much more longer survival time. In animal studies replacement times of 3-10 months have been reported [14], while in humans the glandular epithelium is replaced in up to 3 years [11].

The different cell types of the gastric mucosa release several different products.

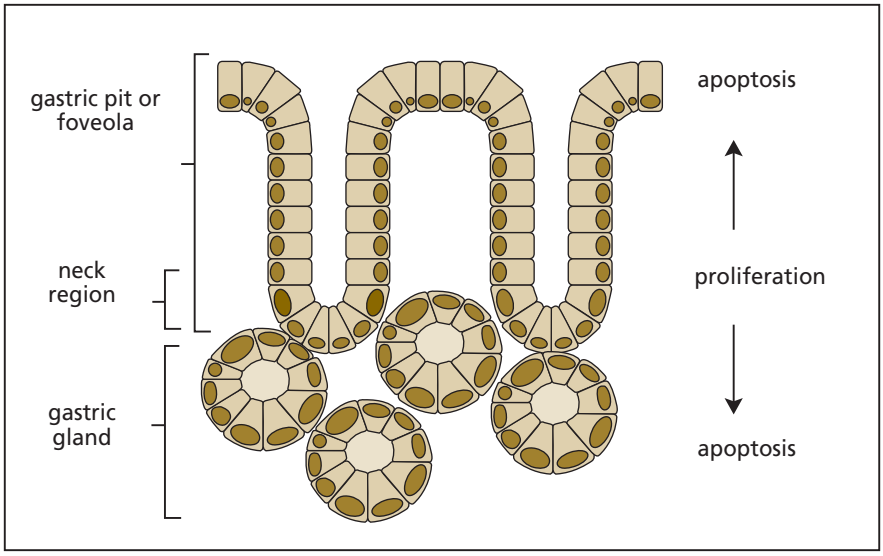

Figure 3. *Upwards and downwards migration and apoptosis of gastric epithelial cells.*

Acid is secreted from the highly specialized parietal cells, located in the corpus of the stomach causing a H^+-concentration in the gastric juice which is 3 million times greater than in blood and tissue. Gastric acid is thought to have two important physiological functions: 1) creating a barrier against infection and 2) activating the digestion of food and facilitating the absorption of dietary components such as calcium en iron. The "gastric bactericidal barrier" is due to low pH, as other constituents of the gastric juice seem to contribute little to the barrier function [16]. A complex system of endocrine cells and neurones interact to control the secretion of acid. Gastrin, histamine and acetylcholine represent physiologically important signals, however, the way they interact to stimulate gastric acid secretion is still debated. Nevertheless, it seems generally accepted that gastrin acts mainly by releasing histamine from so-called ECL-cells by activating gastrin receptors and that histamine stimulates the histamine-2 receptor on the parietal cell.

Gastrin is synthesized and released into the bloodstream by gastrin cells in the antrum. The gastrin cell is an open type endocrine cell with microvilli on the apical membrane of its luminal surface allowing the cell to sense the luminal content, including the H^+-concentration. H^+ ions have an inhibitory effect on gastrin cell activity at pH<4, an increase in gastric pH leads to a decrease in gastrin cell inhibition and an increase in gastrin release.

In addition to gastric acid, parietal cells produce intrinsic factor, an essential protein in erythropoiesis, which is necessary for the absorption of vitamin B_{12} in the small intestine.

Chief cells are present in the glandular layer of the corpus mucosa and synthe-

size 2 major groups of pepsinogens: pepsinogen A and pepsinogen C. These pepsinogens are stored in granules. When physiological stimuli reach the cell, these pepsinogens are secreted in the gastric lumen, where they, at low pH, are converted into pepsin, which has proteolytic activity. Serum levels of pepsinogen can be used as markers for peptic secretion. In most gastrointestinal pathologies the ratio between pepsinogen A and pepsinogen C decreases [17].

Serological gastric biopsy

Pepsinogens

Historical Background [18,19]

The first studies on proteolytic enzymes of the stomach were made in 1785 by Spallanzani, who showed that meat could be dissolved outside the body by gastric juice. Subsequently in 1836, the German physiologist Theodor Schwann introduced the name "pepsin" to designate the most active substance of gastric juice, long before the name "enzyme" was coined. Over the following 60 years, pepsin was considered the prototype of "unorganized ferments" as distinct from the "organized ferments" responsible for such processes as the fermentation of sugar by yeast. In 1930 John Howard Northrop described the crystallization of pig pepsin and demonstrated that enzymes are proteins.

Another important discovery made in this field before 1900 was the observation by John Newport Langley that a slightly alkaline extract of gastric mucosa contains a material (called pepsinogen) that is converted to pepsin by acidification of the extract. In 1938 Roger Herriott described the crystallization of pepsinogen and made possible the incisive study of its conversion to pepsin. Pepsins and pepsinogens were soon implicated in ulcerogenesis. Howes reported in 1936 that, although acid alone failed to prevent the healing of feline gastric ulcers, pepsin and acid together did. In 1942 Schiffrin and Warren found that cat intestinal segments filled with acid did not ulcerate until luminal pepsin was added.

However, only during the last 25 years the structure-function relationship of gastric proteinases and their zymogens has been elucidated. In particular the painstaking work of Taylor on the different forms of pepsin and that of Samloff who studied thoroughly the biochemistry and the pathophysiology of pepsinogens opened the way to the introduction of measurements in clinical practice. Thus, it now seems possible to define the clinical usefulness and the role of pepsinogen measurements in the diagnosis and monitoring of gastric diseases [20].

Pepsinogens in health and disease

Pepsinogen, the inactive precursor of pepsin, and hydrochloric acid are the major secretory products of the gastric mucosa *(Table 1)*. Pepsinogen is the main acid as-

Table 1. *Cells within gastric glands and their secretory product(s).*

Gland area	Cell(s)	Secretory Product(s)
Cardiac	Mucous	Mucus, pepsinogen (Group C)
	Endocrin	See •
Oxyntic	Parietal (oxyntic)	HCl, intrinsic factor
	Chief	Pepsinogen (group A and C)
	Mucous neck	Mucus, pepsinogen (group A and C)
	Enterochromaffin	Serotonin
	Endocrine	See •
Pyloric	Mucous	Mucus, pepsinogen (group C)
	G(astrin) cell	Gastrin
	Enterochromaffin	Serotonine
	Endocrine	See •

- Cardiac, oxyntic and pyloric glandular mucosas contain at least nine different types of endocrine cells. In some of these cells the hormonal product has been identified. Examples include D cells (somatostatin) and A cells (gut glucagons).

partic protease in the stomach and can be found in mucous cells of cardia glands, in chief and mucous neck cells of oxyntic glands and in mucous cells of pyloric glands. In addition, pepsinogen is present in mucous cells of duodenal Brunner's glands. A number of studies have shown, that the seven known pepsinogens (Pg1 through Pg7) can be separated into two distinct groups according to their different patterns of anatomic distribution and cellular origins [18] and most importantly, according to a difference in their immunologic identity [18,21-23]. According to the difference in their mucosal distribution and immunochemical identity, Pg1 through Pg5 constitute one group called pepsinogen A, and Pg6 and Pg7 constitute the second group called pepsinogen C. The majority of the pepsinogens are secreted into the lumen of the stomach where they are metabolized into an active pepsin. A small proportion of the pepsinogens leak for one reason or another into the blood circulation.

Both group A and C pepsinogens can be detected in the blood by radioimmunoassay [24,25]. Pepsinogens and HCl are secreted into gastric juice in response to much the same stimulants. Because group A pepsinogens are present only in oxyntic glandular mucosa, there is a relation between serum group A concentrations and maximal acid secretion [26]. There is general agreement that increasing severity of atrophic gastritis of the fundic gland mucosa is associated with a progressive decrease in gastric pepsinogen and acid output [27,28]. Yamamoto has reported that the

decrease in total pepsinogen output in atrophic gastritis is characterized by a greater decline in pepsinogen A than in pepsinogen C and, therefore, by a decrease in the rate of pepsinogen A-C in gastric juice [27]. This finding is consistent with the histologic features of atrophic gastritis, a loss of pepsinogen A and C secreting chief cells and an increase in pepsinogen C secreting pyloric glands due to pseudopyloric metaplasia. For this reason, the determination of pepsinogen A and C may aid in the screening and identification of patients with an increased risk of gastric cancer [28,29].

Gastrin

Historical background [1,4-6]

A century has passed since the gastric hormone gastrin was first described by John Sidney Edkins as a substance present in extracts of the antral mucosa which stimulates gastric acid secretion. More than a quarter of a century later Simon Komarov in 1938 succeeded in identifying the antral hormone gastrin as a chemical entity separate from histamine. In 1962 gastrin was isolated and characterized by Gregory and Tracy as a pair of heptadecapeptides amides. By about 1970, several groups, including the discoverers of radioimmunoassay (RIA), Yalow and Berson, had independently developed RIA systems for plasma gastrin and these systems form the basis of present day assays for plasma gastrin.

Biological acitivity of gastrin

Gastrin is a regulatory peptide with both endocrine and neurotransmitter function.

The central role in regulation of gastric acid secretion is demonstrated by the marked reduction in acid secretion produced by administering gastrin receptor antagonists [30]. The hormone is produced by the G-cells, which are situated within the glands present in the antral region of the stomach *(Fig.4)* [31]. These cells have microvilli on their luminal surface, allowing them to sense luminal conditions. Protein ingested in the diet stimulates the G-cells to release gastrin into the system circulation. The hormone then stimulates the proximal body region of the stomach to secrete acid. Apart from the antral G-cells there is a source of gastrin production in the duodenum, in the proximal part of the jejunum [32,33] and perhaps in the pancreas [34].

Under fasting conditions, the concentration of gastrin in the serum is approximately 25 pmol/l. Following the ingestion of a protein-containing meal, the serum gastrin level rises 2-3 fold within 15-30 minutes of starting the meal and remains elevated for 1 or 2 hours. There are two main forms of gastrin present in the serum, the 17 amino acid peptide referred to as gastrin-17 (G-17) and the 34 amino acid peptide, gastrin-34 (G-34).

Under fasting conditions, G-34 is the predominant form present in the serum. However, following a meal, the increased gastrin released is mainly G-17, and this becomes the predominant form in serum post-prandially [35].

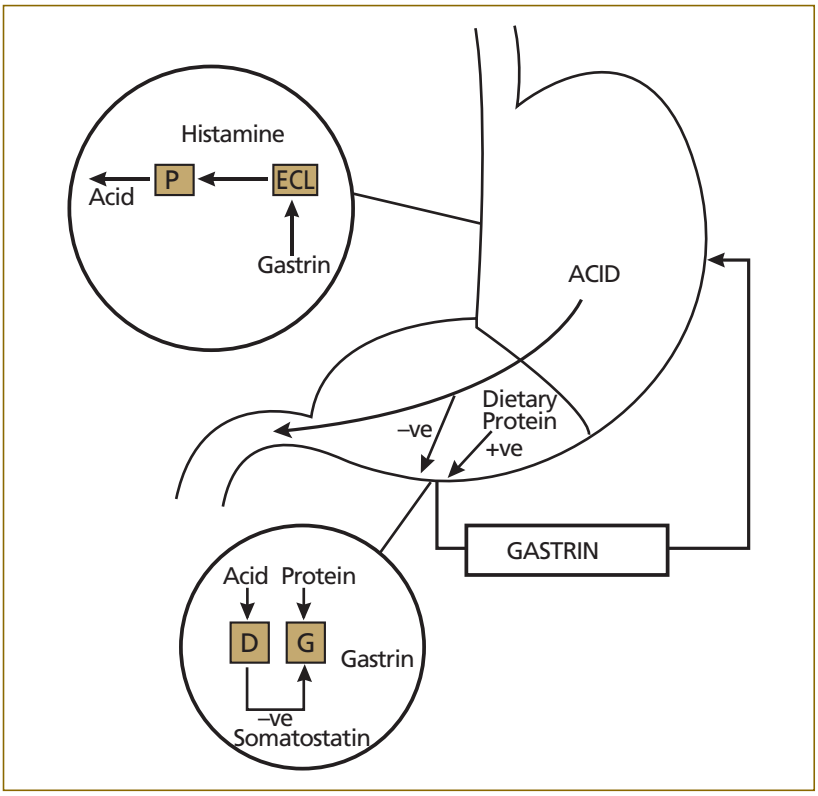

Figure 4. *Role of gastrin in the regulation of acid secretion. Protein components of food stimulate the G-cells in antral mucosa to release gastrin. The hormone circulates and stimulates the body region of the stomach to secrete acid. This occurs via gastrin stimulating the release of histamine from the enterochromaffin-like (ECL) cells, the histamine then activating the histamine-2 receptors on the acid-producing parietal cells (P). The over-production of acid is prevented by a low antral pH inhibiting gastrin release. This inhibitory control is mediated via the release of somatostatin from D-cells situated in close proximity to the G-cells*[31].

The acid-secreting cell of the stomach is the parietal cell, which is found in the gastric glands or pits of the body region of the stomach *(Fig.4)*. The G-cells and the parietal cells are therefore found in different regions of the stomach, the parietal cells being confined to the proximal body region of the stomach and the G-cells to the distal antral region. Gastrin stimulates acid secretion not by acting directly on the parietal cells but by acting on the enterochromaffin-like (ECL) cells, which are situated in close proximity to the parietal cells. These ECL cells have gastrin receptors that cause the cell to release histamine when activated by gastrin. The histamine released in this way then acts on histamine-2 receptors on the adjacent parietal cells causing them to secrete acid.

In addition to stimulating acid secretion in the above way, gastrin also exerts trophic effects on the acid-secreting mucosa of the body of the stomach. The trophic effect of gastrin is most evident on the ECL cells of the oxyntic mucosa. Producing animal models void of gastrin or gastrin receptors results in marked depletion and hypoplasia of the ECL cells [36,37]. The administration of gastrin receptor antagonists produces a similar effect [38]. In contrast, the exogenous administration of gastrin to rats results in hyperplasia and hyperfunction of the ECL cells [39]. Similarly, chronic hypergastrinaemia induced by proton pump inhibitor (PPI) therapy in humans or animals results in hyperplasia of the same cells [40,41]. The trophic effect of gastrin is not confined to the ECL cells but also effects the parietal cells and surface epithelial cells of the oxyntic mucosa [42]. However, the trophic effect exerted by gastrin on some cells may be indirect as they do not all have gastrin receptors. ECL or parietal cells may release heparin-binding epidermal growth factor-like growth factor when their gastrin receptor is activated, and this growth factor may mediate trophic effects on the epithelial progenitor cells [42].

The release of gastrin by the G-cells in the antral mucosa is inhibited by low antral luminal pH [43]. This serves as an important negative feedback control of gastric acid secretion. The role of intraluminal acidity in controlling gastrin release is clearly demonstrated by the exaggerated gastrin respons seen during PPI-therapy [44]. These powerfull acid inhibitory drugs elevate intragastric pH and thus remove the acid-mediated inhibition of gastrin release.

The acid inhibition of gastrin release is mainly mediated by the release of somatostatin from the D-cells situated close to the antral G-cells [45,46], *(Fig.4)*. These D-cells are of the open type and have microvilli enabling them to sense luminal pH. Low intragastric pH stimulates the D cells to release somatostatin, which then exerts a paracrine inhibitory influence on the adjacent G-cells.

Value of gastrin radioimmunoassays in differential diagnosis of gastrointestinal-related diseases

The first application of plasma gastrin RIA was in the diagnosis of gastrinoma. These tumours may be sporadic or may occur on a background of multiple endocrine neoplasia type 1 (MEN-1) [47]. The primary feature of hypergastrinaemia in gastrinoma is that it occurs in the face of acid hypersecretion. Because acid normally inhibits the G-cell, there is also increased circulating gastrin in subjects with reduced or absent gastric acid secretion e.g. chronic atrophic gastritis, especially auto-immune atrophic gastritis, and in some patients on long-term proton pump inhibitors [48]. If achlorhydria is in doubt, an elevated serum gastrin concentration confirms the diagnosis [4].

A reduced plasma gastrin respons after selective antral stimulation points to atrophy of the antral mucosa [49,50].

Gastritis serology

Helicobacter pylori antibodies

The recognition that half or more of the world's population is infected with an organism causing virtual life-long gastritis must rank it one of the most fundamental discoveries in gastrointestinal pathology of the last century. The discovery of *Helicobacter pylori (H. pylori)*, now more than two decades ago [51,52], completely changed our concepts of both gastroduodenal disease and the immunobiology of the stomach.

It has become clear that the bacterium is an important cause of chronic active gastritis [53,54] and an important causative factor in peptic ulcer disease [55]. *H. pylori* is also linked to gastric mucosa associated lymphoid tissue (MALT) lymphoma [56] and to the chain of events leading to gastric carcinoma [57,58].

Infection of the gastric mucosa with *H. pylori* bacteria results in a strong specific local and systemic immune respons [59]. In an attempt to eradicate *H. pylori*, host immune mediators are drawn to the site of inflammation, but are unable to eliminate the bacteria [60]. In humans and mice, *H. pylori* infection stimulates strong specific IgG and IgA antibody production in serum and in the gastric mucosa. The presence of anti-*H. pylori* IgG antibodies in human sera is one of the simplest methods of detecting current or previous *H. pylori* infection. Although a wide variety of serological methods for detection of *H. pylori* have been described in literature, most tests available commercially are enzyme-linked immunosorbent assay methods.

The predictive value is dependent on the sensitivity and specificity of the test and on the prior probability (prevalence) of *H. pylori* infection. This prevalence might differ among patients born in the Netherlands and immigrants, who usually originate from countries with endemic *H. pylori* infections [61]. If the likelihood of *H. pylori* infection is low, the predictive value of a positive test is low. Therefore, an argument can be made that antibody tests should be used only as screening tests. Serological methods have proven especially valuable in screening large numbers of individuals in epidemiological studies [62].

Differences between hosts, their environment as well as differences between *H. pylori* isolates determine disease outcome [63,64]. Still, the majority of infected individuals (80-90%) does not develop disease, and co-evolution of *H. pylori* with their hosts enables a life-long colonization. Changing conditions in the human gastric mucosa may alter gene expression and/or result in the outgrowth of more fit *H. pylori* variants. As such, *H. pylori* is a highly flexible organism that is optimally adapted to its host [64]. The heterogeneity in *H. pylori* populations make predictions on *H. pylori*-related pathogenesis difficult.

Despite falling prevalence rates in the developed world, *H. pylori* is still present in Western Europe and is particularly prevalent among racial minorities and recent immigrants. Identification and eradication of *H. pylori* improves outcomes in patients with peptic ulcer diseases and causes tumor regression in patients with

MALT lymphoma. It is uncertain whether *H. pylori* eradication will improve outcomes in patients with gastric cancer [65].

Gastric autoimmune antibodies

The stomach can fall, like other organs, victim to immunological mechanisms in the pathogenesis of gastric damage [66]. Autoimmune diseases result from inappropriate responses of the immune system to self antigens. It is a question of a disturbed balance between tolerance and aggression. The aetiology of autoimmune diseases remains largely unknown but candidate aetiologic factors include genetic susceptibility and environmental factors.

The pathologic process associated with autoimmune gastritis appears to be directed toward the gastric parietal cells. The pathologic lesion is restricted to the parietal-cell-containing fundus and body regions of the stomach. Parietal cells are lost from the gastric mucosa, and autoantibodies to parietal cells and to their secretory product, intrinsic factor, are present in the serum and in gastric juice. The complement-fixing, precipitating parietal cell antibodies are directed against H^+/K^+-ATPase [67] and, like many organ-specific antibodies, are lacking in species specificity. Many patients with autoimmune atrophic gastritis, almost half, also have analogous antibodies against microsomal antigens of thyroid and, conversely, there is high incidence of parietal cell antibodies in patients with thyroid disease, particularly in those with Hashimoto's thyroiditis [68].

Parietal cell antibodies are also found in a majority of patients with chronic gastritis and appear in from 5 to 10 per cent of normal people, the incidence increasing with age; most such "normal" people with parietal cell antibodies do in fact have chronic gastritis. In addition, when a patient with parietal cell antibodies also develops serum antibodies that appear to be directed specifically against human intrinsic factor, he will be afflicted with pernicious anaemia [67].

Atrophic gastritis as a cancer-prone lesion

The introduction in 1949 by Wood and colleagues in Melbourne [69] of the flexible gastric biopsy tube allowed for the first time study of multiple samples of gastric mucosa from living patients, correlation with other indices of gastric function, and repetitive studies over time.

The histological approach to gastritis, especially the chronic forms, has undergone a series of re-evaluations by different experts in the second half of the last century.

Before the introduction of the Sydney Classification, atrophic gastritis was topographically divided into three types, type A (corpus / autoimmune), type B (antrum / non-autoimmune), and type AB (mixed type) [70,71].

- Type A atrophic gastritis is located in the body and the fundus of the stomach and

is associated with achlorhydria or severe hypochlorhydria. Type A chronic gastritis is characterized by circulating auto-antibodies directed against intrinsic factor and H+/K+-ATPase, which is present on parietal cells in the oxyntic mucosa. Type A autoimmune atrophic gastritis can lead to pernicious anaemia [86]. Type A gastritis is associated with low serum pepsinogen A concentrations and high serum gastrin concentrations, the latter resulting from hyperplasia of gastrin-producing cells. *H. pylori* colonization is uncommon in type A gastritis [72].

- Type B atrophic gastritis is located primarily in the antrum of the stomach, with the possibility of expanding into the corpus, and is associated with normo- or hypersecretion of gastric acid and with peptic ulceration. Thus far, this type of gastritis had been considered idiopathic although irritants, both exogenous, such as hot drinks, spices, alcohol, and tobacco, and endogenous, such as bile reflux, were suggested causes. Nowadays, *H. pylori* infection is thought to be the major cause of type B gastritis [73,74]. Type B atrophic gastritis is associated with reduced plasma gastrin respons to stimulation with food or bombesin. Bombesine is a potent gastrin-releasing stimulus [49,50] and reduced gastrin respons to bombesine can be used as a marker for impaired G-cell function..

- Type AB atrophic gastritis consists of patchy gastritis in both antral and body mucosa, and is associated with an increased incidence of gastric ulceration, dysplasia and gastric cancer. According to Correa [75], a high prevalence of this multifocal gastritis is observed in populations consuming a diet excessive in salty foods and deficient in fresh fruits and fresh leafy vegetables. Patients with this condition have a gradual decrease in hydrochloric acid secretion, and an increase in gastric pH. As a consequence, also this condition can lead to bacterial overgrowth. Autoantibodies to parietal cells are not present.

Classification of chronic gastritis and mucosal atrophy has always been controversial

In an attempt to come to a generally accepted classification, the Sydney System was introduced in 1991. This classification assessed several features of inflammation, atrophy and intestinal metaplasia individually, but reproducibility of individual scores remained rather poor [76]. This Sydney System has been further updated and a visual analogue scale was included to facilitate the grading of the individual features *(fig.5)* (VAS) [77]. In this last paper, atrophy is defined as "loss of specialized glands" [78].

Recently, a reporting system for chronic gastritis in staging and grading was proposed by Rugge and Genta [79]. Staging would convey information on the topography and extension of the gastric atrophic changes, whereas grading should represent the semiquantitative assessment of the combined severity of both mononuclear and granulocytic inflammation. This system could offer gastroen-

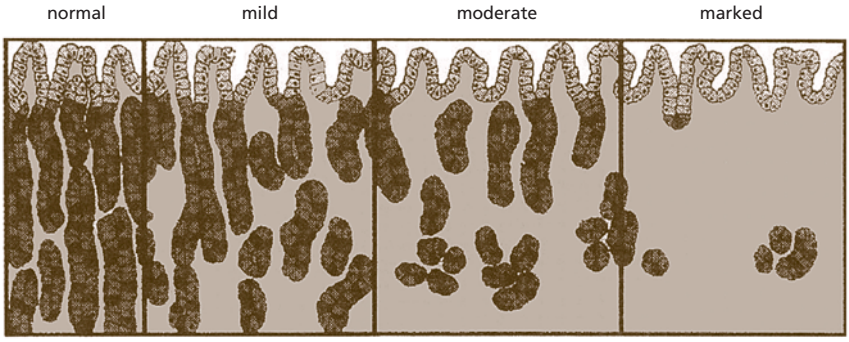

Figure 5. *Visual analogue scale (VAS) for the histopathological grading of gastric corpus atrophy* [77,78]

terologists a more immediate perception of the overall condition of the gastric mucosa while also providing useful information about gastric cancer risk.

The histological changes reported in gastric atrophy are thinning of the mucosa, selective loss of specialized glands and increased spacing between the glands [80]. Furthermore, there should be evidence of replacement of glands by other tissue. It is still unknown, whether fibrosis or inflammatory infiltrate and oedema replace the glands [81].

If the glands are replaced by fibrosis, gastric atrophy may be an irreversible feature. In case of replacement by oedema and inflammatory infiltrate, the gastric mucosa may turn to normal after *H. pylori* eradication. A diagnosis on a single time biopsy remains hazardous.

An increased apoptotic rate may be the explanation of choice for the loss of glands in the development of gastric mucosal atrophy. In normal gastric mucosa, the stem cell number is tightly regulated. If the number of stem cells increases, a fraction of stem cells immediately undergoes apoptosis. On the other hand, if the number of stem cells decreases, stem cell proliferation continues until the normal number is established [13].

In type A chronic atrophic gastritis, a preserved antral mucosa but a profound degree of atrophy in the fundus and body is seen, to such extent that the normal rugae are lost. There is total or subtotal atrophy of specialized glands, with widespread intestinal metaplasia of the surface epithelium. There is loss of normal mucus-secreting cells and replacement by goblet cells and absorptive cells. In the most florid cases, Paneth cells can be present at the base of the pits [82]. Because of the achlorhydria present in type A gastritis there is a compensatory increase in antral gastring secreting-cells (G-cells), with as a result hypergastrinaemia [83].

Pernicious anaemia is mediated by T-lymphocytes like in *H. pylori*-induced chronic gastritis [84]. Judd et al. hypothesized that in pernicious anaemia, this in-

flammatory infiltrate prevents the newly formed cells to differentiate into end-stage parietal cells or zymogenic cells. This would explain the fact that not only parietal cells are depleted, but also the zymogenic population [85].

Type B atrophic gastritis starts in the distal antrum and spreads proximally along the lesser curvature. The degree of atrophy is not as profound as in type A gastritis, and because the disease is mainly antral in distribution achlorhydria is uncommon, and usually there is a normogastrinaemia. Advanced cases of type B gastritis are almost invariably accompanied by intestinal metaplasia; however, in contrast to type A gastritis, the metaplasia is more likely to be patchy.

In type AB atrophic gastritis the process starts at the junction of the antrum and the body of the stomach as independent foci, which then become confluent and spread along the lesser curvature and then to other areas of the mucosa.

Mucosal atrophy is complete when intestinal metaplastic epithelium has replaced the pre-existent gastric glands. Main characteristics of intestinal metaplasia are the intestinal type of epithelium with a brush border and goblet cells, which are easily recognized.

Gastric adenocarcinoma

A century ago, gastric cancer was the most common cancer related cause of death and therefore called the "Captain of Death". Except in China, there has been a decline in incidence in the last 50 years. Nevertheless, gastric adenocarcinoma is still a common cause of cancer death, ranking second worldwide and fifth in The Netherlands, with an incidence of 14 per 100.000 [86]. Of the 2200 newly diagnosed patients each year in the Netherlands about 2/3 is male. The stage of disease at presentation is the most important prognostic factor, and delays in diagnosis remain common. In the Netherlands as many as 50 per cent of patients diagnosed with gastric cancer cannot have a curative operation because of advanced disease. Of the patients who undergo an operation with curative intent, less than 50% survives for more than 5 years due to recurrence.

Fortunately, improved upper endoscopic techniques have made possible not only the discovery of early gastric cancers but also the recognition of mucosal changes that predate malignant degeneration.

The aetio-pathogenesis of gastric cancer is a multifactorial and a multistep process in which both environmental and host-related factors play significant roles [87]. The multistep process stands for the inflammatory cascade of chronic gastritis followed by mucosal atrophy with hypochlorhydria and intestinal metaplasia, dysplasia and finally adenocarcinoma [87-89]. The different steps reflect genetic alterations that drive the progressive transformation of normal cells into malignant

cells. The "precancerous cascade" with atrophic progression, spanning over several decades, has a far-reaching outcome on the functional integrity of the glandular structures of the stomach. Mucosal atrophy is defined as the loss of appropriate number of glands in the gastric mucosa with consequently extinguished secretion of acid and zymogens. The reduction in the area of the fundic gland mucosa appears to correlate with the stepwise reduction in the serum pepsinogen level. Thus, the serum pepsinogen level is considered a reliable marker for the extent of gastric precancerous conditions or lesions [28].

Chronic infection with *H. pylori*, as aetiologic agent of the initiating event of the inflammatory cascade, is a major force driving the precancerous process and recently reports indicate that gastric cancer chemoprevention via eradication of *H. pylori* infection is a viable option [90].

Prevention is the most promising strategy to control the disease. Apart from eradication of *H. pylori* infection, cancer prevention include dietary interventions with antioxidants such as vitamin C or A, and attention for the nutritional status of the host [91].

In countries with high incidence of gastric cancer, such as Japan (90 per 100.000), endoscopic screening programs are cost effective. In Western countries endoscopic screening is not cost beneficial due to low incidence.

In Western countries non-invasive screening programs may be useful to identify risk groups exposed to risk factors for a more rapid development of atrophic gastritis. Serological testing for *H. pylori*-related atrophic gastritis is an aid in clinical decision making, orientating further referral for endoscopical and histological examination.

Summary

Serological gastric profile provides information about the morpho-functional status of the gastric mucosa. A decreasing pepsinogen A and A/C ratio and an increasing serum gastrin are known to reflect an increasing severity of atrophic corpus gastritis. *H. pylori*-related atrophic gastritis and hypochlorhydria are well-documented risk factors for noncardia gastric cancer.

Early serological detection and endoscopical surveillance of achlorhydric atrophic gastritis on the one hand, and reduction of the infection rate of *H. pylori* on the other, may prove suitable means in the prevention of gastric cancer mortality.

Serological investigation at the primary care level of high-risk populations, i.e family members of gastric cancer patients, may provide an opportunity to test and scope this particular group.

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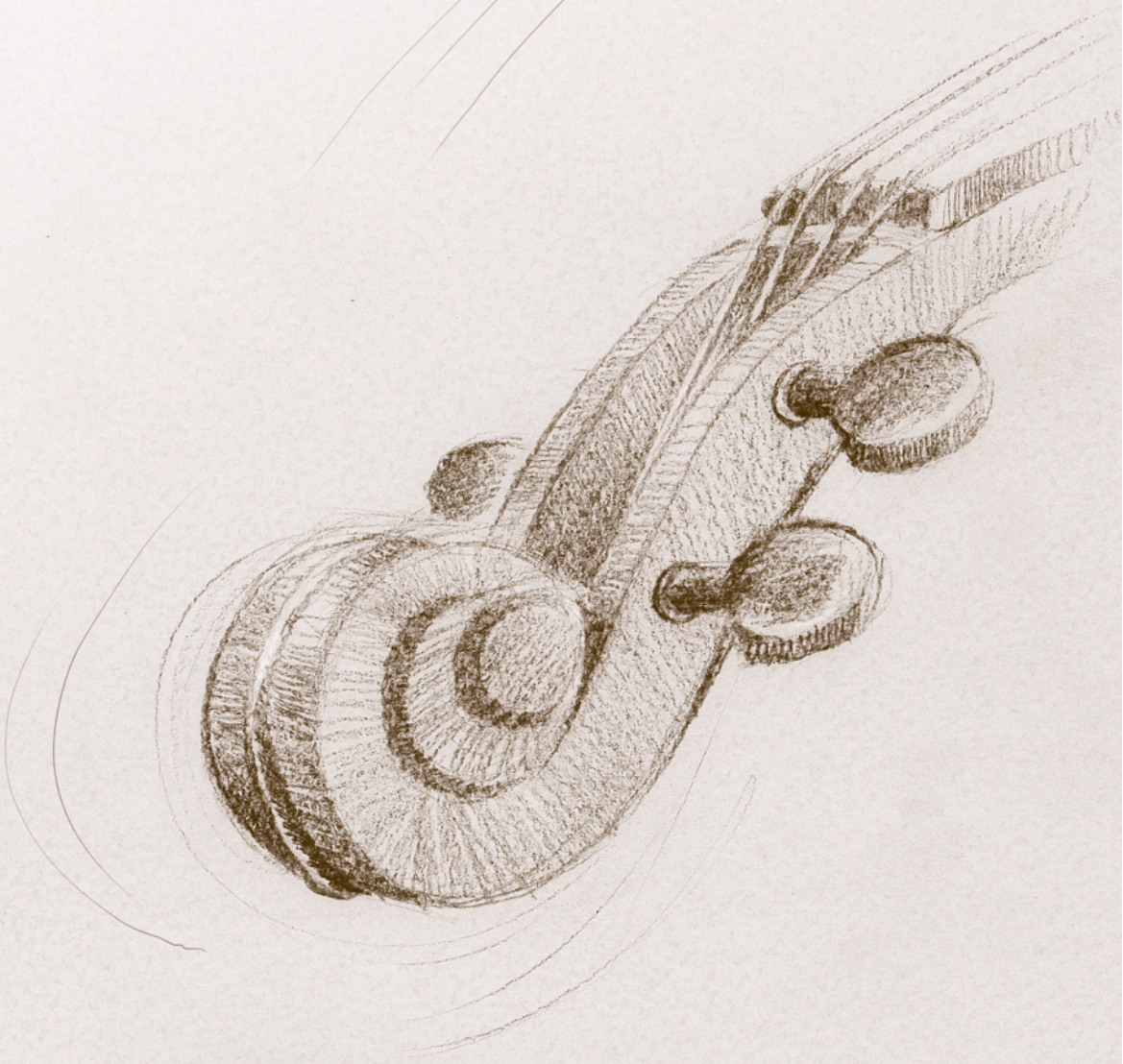
Chapter II



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One cannot be medically literate
without the fluency in the language of science.

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Chapter II

Character, aims and outline of the thesis

Character of the thesis

The character of this thesis is in a way unique that all the studies on atrophic gastritis are performed in an unselected asymptomatic population at the primary care level. The study population is a sample of the inhabitants of several rural villages on Zuid-Beveland, the middle peninsula of the Dutch province Zeeland. This region has a fairly stable resident population with predominantly indigenous people and is therefore an appropriate area for epidemiological investigation. All study subjects were recruited just from one general practice. The studies have an explorative character and no sample size and statistical power calculation was used. It is demonstrated how the general practitioner can carry out studies in his own practice on a scientific base, contributing to a deeper understanding of health care questions. .

Aims of the thesis

The studies included in this thesis aim to contribute to the knowledge in primary care of

1. the seroepidemiological and aetiological aspects of atrophic corpus gastritis in a primary health care community
2. the reliability of ^{13}C-urea breath test for the presence of *H. pylori* infection in patients with atrophic corpus gastritis
3. a novel non-invasive stimulation test for the diagnosis of the degree of gastric body atrophy

A search of the literature on atrophic gastritis will turn up no primary care research activity into this subject. Most of what we know about how to diagnose and survey chronic atrophic gastritis is derived from the expert opinion of specialists and from research generated in secondary and tertiary care setting. Most importantly, secondary and tertiary care research involves selected patient populations for whom the probability of disease and outcome may be different from what we encounter in the frontline primary care practice.

Outline of the thesis

The introductive **Chapter 1** invites the interested and critical reader to take knowledge of the background of the serum profile of gastric mucosa in relation to histological diagnosis of atrophy. This general introductive overview provides essential current information for complete understanding of the research described in this thesis.

Chapter 2 comprises the character, aims and outline of this thesis

In **Chapter 3** the question is investigated whether the gastric serum biopsy might be useful as a diagnostic tool in management of the dyspeptic patient in primary care. We survey the literature on this subject and try to formulate a recommendation for the clinical application of the gastric serum profile as non-invasive first-pass marker of gastric disease.

In **Chapter 4** the seroprevalence of atrophic corpus gastritis, a risk factor for gastric cancer, is assessed in the general Dutch autochtonous population. A community based explorative study by serology is performed in a large primary cohort of 997 consecutive subjects. In addition, the involvement of *H. pylori* infection and gastric autoimmunity in serological atrophic corpus gastritis is studied.

Chapter 5 explores the diagnostic accuracy of gastric serum profile in relation to endoscopical and histological diagnosis of gastric body atrophy. Cancer risk is directly correlated with the severity and extent of mucosal atrophy, making identification of atrophy a goal in cancer prevention programs. The study population comprises asymptomatic subjects with serological atrophic corpus gastritis, selected from the large primary cohort of 997 primary care subjects on the basis of the serum profile of gastric body atrophy.

Chapter 6 evaluates the detection of *H. pylori* infection with ^{13}Carbon urea breath test in subjects with asymptomatic atrophic corpus gastritis. The few available data based on selected patients point to limited value of the ^{13}C-UBT in atrophic con-

ditions of the stomach. However, such information may not be applicable to general practice. In this study the diagnostic accuracy of ^{13}C-UBT for *H. pylori* infection is compared to biopsy, culture and serology in 20 asymptomatic primary care patients with histologically proven gastric body atrophy.

In **Chapter 7** a novel non-invasive stimulation test in the diagnosis of the degree of atrophic body gastritis is presented. Proton pump inhibitors (PPIs) are known to be potent pepsinogen and gastrin releasing drugs. The effect of short-term administration of a PPI on the serum concentration of pepsinogen A and gastrin is evaluated in asymptomatic subjects with serological atrophic corpus gastritis. Our premise is that PPI-stimulated pepsinogen A in subjects with putative gastric body atrophy has an inverse graded relation with atrophy.

In **Chapter 8** we try to gain insight in the possible involvement of *H. pylori* infection in the development of the atrophic gastritis characteristic of pernicious anaemia. Overt pernicious anaemia is usually *H. pylori* negative. The pre-pernicious stage of gastritis would be a desirable period to test for *H. pylori* infection. Therefore the seroprevalence of *H. pylori* infection and atrophic corpus gastritis is studied in first and second degree relatives of patients with pernicious anaemia and compared with the seroprevalence in relatives of patients with hypertension, a disorder which is known to be as a rule not related to *H. pylori* infection.

In **Chapter 9** the results of the various studies presented in this thesis are summarized and commented.

Chapter III

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There must be as many views about the qualities that make a good general practitioner as there are patients.

(S. Webster 1986)

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Chapter III

The serological gastric biopsy: a non-endoscopical diagnostic approach in management of the dyspeptic patient

SIGNIFICANCE FOR PRIMARY CARE BASED ON A SURVEY OF LITERATURE

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Keywords: gastrin, *Helicobacter pylori* gastritis, pepsinogens, primary care, serological gastric biopsy

Scandinavian Journal of Gastroenterology 2002; 37 Suppl 236: 22 – 26 (modified version)

Abstract

Background: The measurement of the serum concentration of the secretory products of the gastric mucosa, pepsinogen A (PgA), pepsinogen C (PgC) and gastrin is called the *serological gastric biopsy*. Additional measurement of *Helicobacter pylori* antibodies and antibodies to parietal cells and intrinsic factor supports the non-invasive diagnostic value of the serum markers. In many clinical studies the diagnostic potential of the serum markers in predicting the topography and severity of gastric mucosal disorders has been established.

Aim: To assess the diagnostic value of the *serological gastric biopsy* for primary care.

Method: Survey of literature.

Results: The cell-physiological background of the *serological gastric biopsy*, the interpretation of the outcome of serum markers and the relation of these parameters to various gastric mucosal disorders is described.

Measurement of PgA gives a reliable possibility to discriminate between mucosal gastritis and functional dyspepsia. PgA is raised in duodenal, gastric, and pyloric ulcer even though gastrin is normal. Both PgA and gastrin are raised in renal insufficiency and the Zollinger-Ellison syndrome. A low PgA is indicative of mucosal atrophy and a good indicator for gastric hypoacidity. An additional low PgA:C ra-

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tio is indicative of atrophic gastritis or extensive intestinal metaplasia of the stomach. A hypopepsinogenaemia can also be an alarmsymptom for gastric cancer. A low PgA and a high gastrin is indicative of corpus atrophy.

Conclusion: In primary care the *serological gastric biopsy* might be a feasible and appropriate diagnostic method for management of the dyspeptic patient.

Further research in general practice has to be done to validate the predictive value of the *serological gastric biopsy* and to define a diagnostic strategy.

With due attention to guidelines [1], the diagnostic and therapeutic strategy for dyspepsia in general practice remains an individual approach. The medical goal is to make an appropriate diagnosis, to give curative therapy and also to protect the patient against unnecessary investigations. Open-access endoscopy has been proven to be a valuable service to primary care, greatly enhancing the diagnostic accuracy in dyspeptic patients entering primary care [2,3].

It is a challenge for the general practitioner to make a good pre-endoscopic selection, based on validated predictors of organic causes for dyspeptic symptoms. In various primary care and also clinical studies the used predictive factors, like subgrouping of symptoms and clinical judgement, appear to be not sufficiently effective [4-8]. However, an upper endoscopy as invasive test, despite of its high diagnostic reliability, is burdensome and stressing for the patient and not the least expensive for health care. Moreover, the effectiveness of prompt endoscopy above empirical therapy for dyspepsia in primary care is not proven [9].

Previous clinical studies have emphasised the possible usefulness of pre-endoscopic screening for IgG antibodies against *Helicobacter pylori* in dyspeptics to avoid unnecessary endoscopies [10,11]. However, using *H. pylori* serology with a well-defined strategy in general practice has not yet been validated. In the total group of dyspeptic patients in primary care, *H. pylori*-testing has no value in addition to history-taking in the diagnosis of peptic ulcer disease [12]. Validated parameters are necessary in a pre-endoscopic approach with an effective sensitivity and specificity to eliminate or to ascertain the suspicion of gastric diseases.

In various gastroenterological clinical studies it has been established that the secretory functions of the gastric mucosa can be used for diagnostic purposes. Measuring the serum level of pepsinogen A, pepsinogen C and gastrin may contribute to a reliable non-invasive histological assessment of the state of the gastric mucosa [13-15]. This specific non-invasive approach, known as the *serological gastric biopsy*, might be very attractive to use also in primary care.

In daily practice the most important gastric organic diseases are the various types of gastritis (chronic superficial and chronic atrophic), peptic ulcers and rarely gastric cancer [2].

With few exceptions (e.g. in patients with autoimmune chronic corpus gastritis), gastritis is associated with the presence of *H. pylori.* Inflammation and atrophy

of the gastric mucosa result in impairment of gastric secretory functions (e.g. secretion of gastric acid, pepsin and gastrin). In all these organic disorders the serological gastric biopsy can be used to make a pre-endoscopic diagnosis in accurately detecting which patients will most benefit from gastroscopy.

Cell-Physiological Background of the Serological Gastric Biopsy

The gastric mucosa has a highly differentiated cellular structure with various mucosal glands, each with a specific function related to specific glandular products. The most important gastric glandular products are *pepsinogen A, pepsinogen C* and *gastrin* [16,17]. Pepsinogen is the proenzyme of pepsin; pepsin digests dietary proteins at low pH. Pepsinogen A (PgA) and pepsinogen C (PgC) are produced by the chief cells of the fundic mucosa; PgC is also found in the pyloric glands of the antrum and in Brunner's glands in the duodenum[18]. Gastrin is a regulatory peptide hormone that excites the secretion of acid and gastric juice. Gastrin is mainly produced by G(astrin)-cells in the antral mucosa and at a lower rate by G-cells in the duodenum. Gastrin is secreted directly in the circulation [19-21]. PgA and PgC are excreted into the gastric lumen and enter the circulation via the blood/mucosa barrier [22].

Serum Markers and Their Interpretation

In general one can say that the level of the serum markers can be interpreted like a barometer for the weather in the stomach. In the interpretation of the serum concentration of pepsinogens and gastrin there are several anchorpoints:

1. An increase in serum concentration reflects more functional cell mass, like hyperplastic or hypertrophic mucosal conditions [13].
2. A decrease in serum level reflects a reduction in cell mass, for example in atrophic mucosal conditions or in the case of partial gastrectomy [13].
3. Disturbance of the integrity of the barrier between the gastric mucosa and circulation in the case of inflammation gives a clear increase in the serum concentration of pepsinogens because of the about 24000 fold higher pepsinogen concentration in the gastric fluid than in the circulation [13,22].
4. The serum concentration of PgA and PgC has to be interpreted in the light of the topology of the producing cell sources. Because of the additional PgC production in pyloric antrum glands and in duodenal Brunner's glands there might be a different serum level of PgC compared to PgA in various gastric disorders. The interrelation between PgA and PgC, expressed in the PgA/C ratio has a separate diagnostic value [13].
5. Furthermore, the concentration is influenced by common factors such as age (very young persons have low pepsinogen levels), fasting (eating increases gastrin serum level), smoking habits (smokers have significantly higher pepsinogen A levels than non-smokers) and chronic renal failure (healthy kidneys extract pepsinogens out of the circulation)[13,20,22,23].

Immunological Gastric Serum Markers

A special chapter in the use of serological diagnostic investigation of the gastric mucosa is the phenomenon of the immune response in the stomach. Patients with a *H. pylori* infection of the stomach develop a serum immune response consisting of the IgG and IgA immunoglobulin types. Measuring of those antibodies is indicative of exposure to *H. pylori* infection of the gastric mucosa and enables the diagnosis of *H. pylori*-associated gastritis [11]. Adding serum recognition of the cytotoxin associated gene A (CagA) and the vacuolating cytotoxin (VacA) proteins further refines diagnostic accuracy [24]. Moreover, patients with antibodies against parietal cells and intrinsic factor have antigastric autoimmunity [25]. So co-measurement of the immunological markers does complete the overall serological gastric biopsy.

Serum Markers in Relation to Gastric Mucosal Disorders

Many clinical studies have validated measurement of the serum markers [26] and have established its clinical relevance with respect to diagnosis and follow up[13,27,28]. A great advantage of the serological gastric biopsy is the fact that it gives a reflection of the histological condition of the whole stomach; there is not the problem of a sampling error. To take full profit of the results of the serological biopsy it is advisable to give attention to the topography of the stomach:

Corpus-predominant gastric atrophy:

Corpus limited advanced atrophic gastritis is an outstanding example of the diagnostic reliability of the serological gastric biopsy. Various studies have established the serological profile of corpus atrophy [28-31]. First of all the serum level of PgA is (very) low because of atrophy of the chief cells in the corpus; there is a normal or mild decreased PgC, because of the escape production of PgC in antrum and duodenum; the serum level of gastrin is (very) high because of a functional intact antrum and the antral drive to activate acid production in the atrophic or lost parietal cell mass. The Pg A/C ratio is low because of a (very) low PgA and a relative normal or less reduced PgC.

Autoimmune antibodies to parietal cells and intrinsic factor often appear to be positive. Antibodies against *H. pylori* are frequently negative. Severe gastric corpus atrophy is the aetio-pathogene background of pernicious anaemia due to loss of glandular tissue and lack of intrinsic factor [25]. Gastric corpus atrophy is also a precursor lesion of gastric cancer [32,33].

Early detection of gastric corpus atrophy is important for prevention of the consequences of vitamin B_{12}-deficiency and for further endoscopical analysis and surveillance of the preneoplastic atrophy.

Antrum-predominant gastric atrophy:

Antrum limited advanced atrophic gastritis, a relatively rare gastric disorder, has a recognizable serological pattern. The loss of antral glands results in reduction in

the number of G-cells which are the main source of the circulating gastrin, particularly gastrin-17 (G-17). It is conceivable that this loss results in a measurable impairment of the antral mucosa to physiological stimuli which also leads to low output into the circulation. Some previous observations indicate that this is, indeed, the case [34]. So, a low raise of G-17 serum level after a test meal is an indicator of antrum atrophy, whereas the PgA and PgC levels remain slightly subnormal. Advanced antral gastric atrophy is also a premalignant condition and the early recognition of this disorder has a preventive goal [32,33]. In contrast to the corpus limited gastric atrophy the antrum limited atrophy has probably no autoimmune background, so autoantibodies to parietal cells and intrinsic factor are negative. *H. pylori* antibodies often appear to be positive.

Multifocal gastric atrophy:
The occurrence of advanced atrophic gastritis multifocally in the stomach (pangastritis) gradually impairs all secretory functions. So, PgA and PgC are low, the ratio PgA:C is low (PgC can still be produced in the duodenum); serum gastrin is low and cannot be stimulated. There is an increased risk of gastric neoplasias [32,33].

Chronic active gastritis and peptic ulcers:
After the rediscovery of *H. pylori* (initially named Campylobacter pyloridis), it became clear that this organism represents the main causative agent of gastritis [35,36]. Inflammation of the gastric mucosa impairs the integrity of the barrier between mucosa and circulation. Hypergastrinaemia and hyperpepsinogenaemia are secondary to *H. pylori* infection and are related to the extent of mucosal inflammation [37,38]. Active gastritis and peptic ulceration have a far identical serological profile. Peptic ulcers have often a relative low PgA/C ratio because of a higher PgC serum level compared to PgA. Eradication of *H. pylori* results in a significant fall in serum gastrin and in pepsinogen A and C concentration. Especially serum pepsinogen C appears to be useful in monitoring the treatment outcome in patients with *H. pylori*-associated gastritis [39].

Gastric cancer
Cancer of the gastric mucosa and its precursors can be serologically suspected in case of a low PgA and a low PgA/C ratio. The significance of a low serum PgA/C ratio is higher than a low serum PgA level in those patients [31,40,41]. *H. pylori* antibodies can be either positive or negative.

Use of proton pump inhibitor (PPI) drugs
PPI's are inhibitors of gastric acid secretion with a long duration of action and exert their effect by a non-competitive antagonism of the H^+, K^+-ATPase in the parietal cell [42].

It is therefore understandable that PPI's have an effect on the serum concen-

tration of gastrin and the pepsinogens. Gastrin serum levels increase because of inhibited acid secretion. Serum PgA and PgC levels rise because of increased storage and release of PgA into the circulation as response to inhibition of exocrine secretion in the gastric lumen. The pepsinogen A:C ratio is not affected since the levels increase in parallel [43].

Remainder of gastric mucosal disorders.

Typical patterns of the serological markers are found in patients with the Zollinger-Ellison syndrome, in patients with hypertrophic gastropathy and in patients with partial gastrectomy [13]. The first two are rare disorders in general practice and are mentioned here just for purpose of illustrating the patho-physiological background of the serological gastric biopsy.

In the *Zollinger-Ellison syndrome* due a gastrin producing gastrinoma there is a high level of serum gastrin [44]. Because of the positive correlation with gastric acid output the pepsinogen serum level will rise resulting in hyperpepsinogenaemia. Furthermore the pepsinogen A:C ratio is high due to a higher A level because of the trophic effect especially on the oxyntic mucosa. Since synthesis of PgA is limited to the oxyntic mucosa, a relatively larger contribution of PgA compared to PgC could be expected in these patients [13,44].

Ménétrier's disease, hypertrophic gastropathy, is characterised by overgrowth of the surface epithelium, formation of mucosal cysts, and occasional appearance of pseudopyloric metaplasia. This disease is well known as a precancerous condition. Patients with a hypertrophic gastropathy have a raised level of both serum PgA and PgC whereas the PgC level is significantly more increased. So the PgA:C ratio is relatively low. Whether this low ratio is caused by hyperplasia of the mucous neck cells in the gastric body or inflammation of the antrum remains to be established. The serum gastrin level is also elevated [13,27].

In *partial gastrectomy* the whole antrum and a large part of the gastric body have been removed, thus the gastrin and a substantial part of the pepsinogen A and C producing mucosa are gone. Postoperative atrophy of the gastric remnant will also cause depletion of pepsinogen A producing cells. Thus meal stimulation reveals lack and shortage of synthesis capacity of gastrin and both pepsinogens. (13,26).

Finally, in *table 1* a simplified schematic overview shows the behaviour of the serum markers in different gastric disorders.

Consideration and Conclusions

"Nothing is impossible although some things are improbable" is an appropriate working slogan for the general practitioner especially in management of the dyspeptic patient. Most dyspeptic patients in primary care are managed without confirmatory

Table 1. *Simplified schematic overview of serum markers in different gastric disease states*

	PgA	PgC	A/CRatio	Gastrin	AntiHp	AntiPC
Corpus predominant gastric atrophy	↓	n	↓	↑	– / +	+
Antrum predominant gastric atrophy	↓	n	↓	↓	+	–
Multifocal gastric atrophy	↓	n	↓	↓	+	–
Chronic active gastritis	↑	↑	↓	↑	+	–
Peptic ulcers	↑	n/ ↑	n/ ↓	↑	+	–
Gastric cancer	↓	n	↓	n/↓	+ / –	+ / –
PPI-medication	↑	↑	n	↑	+ / –	–
Zollinger-Ellison syndrome	↑	↑	n	↑	–	–
Hypertrophic gastropathy	↑	↑	↓	↑	–	–
Partial gastrectomy	↓	n	↓	↓	+	–

investigation. Optimal history-taking remains a sovereign first diagnostic step [12], often followed by symptom-based empirical treatment. Open access facilities for upper gastrointestinal endoscopy has made the diagnostic process in dyspepsia easier. However, only in 18% of patients aged 45 years or less without empiric treatment, climbing to 36% in those with long-term treatment, a relevant endoscopical diagnosis is found. In the age group of more than 45 years, those percentages were 23% and 30% respectively [45]. The discriminative value of the serological gastric biopsy might be a welcome support to select patients for gastroscopy and to augment the percentage of relevant endoscopic diagnoses. However, it should be mentioned that in the case of atrophic conditions of the stomach, gastric serum profile can diagnose atrophic gastritis with high specificity but low sensitivity. Antibodies to *H. pylori* or CagA can diagnose atrophic gastritis with high sensitivity but low specificity. A combination of two tests, e.g. *H. pylori* antibodies and pepsinogen A, may balance this issue and provide adequate diagnostic tools [46].

It can be *concluded* that the determination of the serum concentration of pepsinogen A, and, on indication, measurement of pepsinogen C- and gastrin serum levels have clinical relevance for a number of gastroduodenal disorders and could also have a place in the diagnostic equipment of the general practitioner. Further investigation in primary care is necessary to validate this relevance.

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Chapter IV

◆

Music is the medicine of a troubled mind.

◆

Chapter IV

The role of Helicobacter pylori and autoimmunity in serological atrophic corpus gastritis in a Dutch primary care community

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Keywords: serological atrophic corpus gastritis, primary care community, pepsinogen, gastrin, *Helicobacter pylori* infection, parietal cell antibodies

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Abstract

Background & Aims: Atrophic corpus gastritis predisposes to the development of vitamin B_{12} deficiency and gastric cancer. Little is known about the seroprevalence of atrophic corpus gastritis in primary care communities in Western Europe. The Dutch province Zeeland has a fairly stable resident population with predominantly indigenous people and is therefore an appropriate area for epidemiological investigation. The aim of this study was to investigate the seroprevalence of atrophic corpus gastritis and the involvement of *Helicobacter pylori (H.pylori)* infection and autoimmunity in a West-European primary care community.

Methods: 997 consecutive subjects (455 males and 542 females; mean age (±SD) 52 (±16) years) in a single Dutch general practice were asked to participate in this study by filling in a questionnaire and donating a fasting blood sample. Gastrin, pepsinogen A and C and antibodies to *H.pylori* were measured in serum by well-validated immunological methods. Our validated criteria for serological advanced atrophic corpus gastritis, were a serum concentration of PgA < 17µg/l, a PgA/C ratio < 1.6 and a serum level of gastrin > 100ng/l.

The resultant atrophic corpus gastritis-group was compared to a nested case-control group of subjects without serological atrophy matched for age and sex, ran-

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domly chosen from the total study group of patients. In the atrophic corpus gastritis-group and in the nested case-control group antibodies to parietal cells (anti-PC) and intrinsic factor (anti-IF) were also measured.

Results: 34 subjects (3.4%) fulfilled the serological criteria of advanced atrophic corpus gastritis; 21 (62%) of them compared to 17 of 34 (50%) age- and sex matched nested controls were *H.pylori* positive (n.s.). In the atrophy group 15 subjects had anti-PC (44%) compared to one (3%) in the control group ($p<0.005$). When comparing to the nested case controls the relative risk of having atrophic corpus gastritis is 1.62 (0.62-4.24) for *H.pylori* infection, and 24 (3-201) for anti-PC. In the atrophy group 5 persons had anti-IF (15%) and 2 in the control group (6%; ns). Four of the 5 patients with atrophy who had anti-IF, also had anti-PC. Seven atrophy persons had both *H.pylori* infection and anti-PC; 5 atrophy subjects had neither anti-*H.pylori* nor anti-PC and anti-IF. Nine of the 25 subjects with atrophic corpus gastritis had lowered vitamin B_{12} levels in serum. Persons with atrophic gastritis did not differ from controls regarding the presence or history of gastric complaints.

Conclusions: The seroprevalence of advanced atrophic corpus gastritis in a primary care community in The Netherlands is 3.4%. Both autoimmunity and *H.pylori* infection appear to be relevant for the pathogenesis of atrophic corpus gastritis. When compared to controls the odds ratio of having atrophic corpus gastritis was significantly higher ($p<0.025$) for parietal cell antibodies[24] than for *H.pylori* antibodies (1.62). In view of the decreasing risk of *H.pylori* infection in the Western world, it is likely that the impact of *H.pylori* on the development of atrophic corpus gastritis will further diminish.

Introduction

Atrophic corpus gastritis may lead to vitamin B12 deficiency due to reduced intrinsic factor production[1]. It further predisposes to the development of gastric cancer [1-6].

Little is known about the seroprevalence of atrophic corpus gastritis in primary care communities in Western Europe. Early serological identification of individuals with gastric precancerous conditions followed by endoscopy, would be a desirable aim of a screening program in the general population [7,8].

The province of Zeeland in The Netherlands is a good region to survey with a stable and predominantly indigenous population. The rural inhabitants of this area are a relatively homogeneous group of Dutch people with large families. Zeeland was up to 50 years ago a fairly isolated part of the country in the geographical delta of the river Schelde. This stable population seems to be an ideal target group for epidemiological screening studies in a primary care community.

Screening tests should ideally be convenient, virtually free of discomfort or risk

and economically attractive [9]. Gastric endoscopy with biopsy is a commonly used test for the diagnosis of chronic gastritis but is invasive and as such has none of these characteristics. Its value is further hampered by sampling problems of biopsies. A reliable serological test would be preferable.

The serological gastric biopsy fulfils the above mentioned screening test criteria and might be a reliable diagnostic instrument to identify gastric mucosal disorders [10]. Taking a *serological gastric biopsy* means the measurement of the serum concentration of the gastric secretory products pepsinogen A, pepsinogen C and gastrin. Prior to the discovery of *Helicobacter pylori*, serum pepsinogen levels had been shown to vary with gastric mucosal histology. Samloff demonstrated a progressive fall in serum pepsinogen A (PgA) with increasing degrees of gastric fundus atrophy, reflecting loss of zymogen producing cells. A less marked fall in serum pepsinogen C (PgC) occurs in atrophic corpus gastritis because PgC (unlike PgA) is also produced by pyloric glands of the antrum and in Brunner's glands in the duodenum. These non-parallel changes result in a progressive lowering of the PgA/C ratio with increasingly severe gastric fundus atrophy [10,11]. Very low PgA-levels and a low PgA/C ratio are accurate predictors of severe atrophic corpus gastritis [11-13] and are frequently found in gastric cancer [14-16].

In the setting of general practice the diagnostic value of the gastric serum markers has had no attention up to now, probably because of unfamiliarity with the subject among general practitioners. Therefore our present study was undertaken to evaluate the performance of *the serological gastric biopsy* at the primary care level.

The first aim of this study was to investigate the prevalence of atrophic corpus gastritis by serology in a Dutch primary care community.

The second aim was to determine whether subjects with serological atrophic body gastritis have evidence of *H. pylori* infection and/or gastric autoimmunity.

H. pylori is a major risk factor for chronic gastritis and plays a critical part in the promotion of atrophic gastritis [17]. Gastric autoimmunity plays an important part in the progression of atrophic corpus gastritis after *H. pylori* infection [18] and also independent of *H. pylori* infection [19].

Because *H. pylori*-associated atrophic corpus gastritis is a risk factor for gastric cancer [20-24], it might be clinically important to prevent atrophic gastritis by eradicating *H. pylori*.

Subjects and Methods

Subjects

In a period of 2 years, a total of 997 consecutive subjects, 455 males and 542 females, mean age 52 years (±SD 16 year), was enrolled from the general practice 's-Gravenpolder in The Netherlands. The subjects had made an appointment to consult the family doctor for common medical problems and were asked to par-

ticipate in the study by donating a fasting blood sample and filling in a questionnaire with questions covering upper gastrointestinal symptoms, past gastric diseases, family history of gastric disorders and the use of acid lowering drugs or antibiotics. The minimum age was 18 years while there was no upper age limit. Pregnant patients and patients with gastric resection, renal insufficiency and those using antisecretory agents or antibiotics recently were excluded from the analysis. The study was performed according to the declaration of Helsinki and all patients gave informed consent.

Methods

All serum samples were examined by well-validated radioimmunoassays for pepsinogen A and C and gastrin [25]. *H. pylori* serology was performed by a validated enzyme immunoassay detecting specific immunoglobulin G against a homogenate of 6 strains of *H. pylori*. Western blots of this homogenate showed the presence of CagA bands, indicating a cytotoxic variety of *H. pylori*. The results were expressed as the absorbance index (AI): serum with an AI > 0.32 IgG *H. pylori* antibody was considered evidence of *H. pylori* infection [26].

Our validated criteria for advanced atrophic corpus gastritis, corresponding to pentagastrin refractory achlorhydria or severe hypochlorhydria (PAO < 5mmol/hr), were a serum concentration of PgA < 17 μg/l, a PgA/C ratio < 1.6 and an accompanying serum concentration of gastrin > 100 ng/l [25,27]. Parietal cell autoantibodies were analysed using a commercially available kit (Autoscreen 1, Scimedx Corporation, Denville, NJ 07834, USA). Serum vitamin B_{12} levels were measured by radioimmunoassay.

Subjects with serological atrophic corpus gastritis were compared to an age and sex matched nested case-control group without serological evidence of atrophic corpus gastritis randomly chosen from the total study group of participants [28,29]. In the case of more than one corresponding control subject, one subject was at random chosen.

Statistical analysis

Results were analysed using Chi-square test with Yates correction. The relative risk, its 95 percent confidence interval and comparison of different relative risks were performed using standard statistical methods [30].

Results

Atrophy findings

34 (3.4%) of the 997 subjects fulfilled the serological criteria of advanced atrophic corpus gastritis, of which 19 were female (56%). The mean age in the atrophy group was 67 years (range 28-91) compared to 52 years (range 18-91) in the total group *(Figure 1)*.

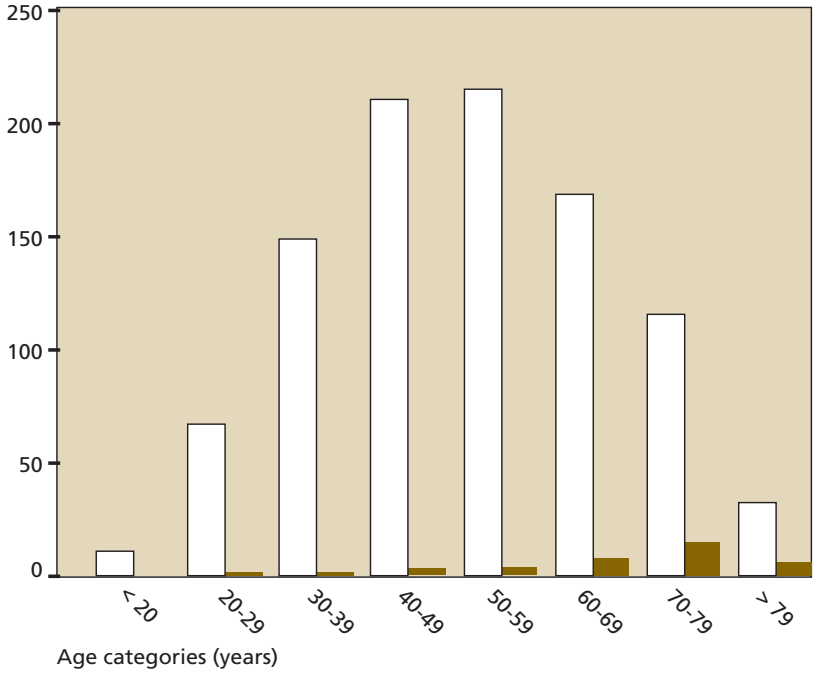

Figure 1. *Age distribution of 34 subjects with serological atrophic corpus gastritis (filled bars) compared to the 963 subjects without (open bars).*

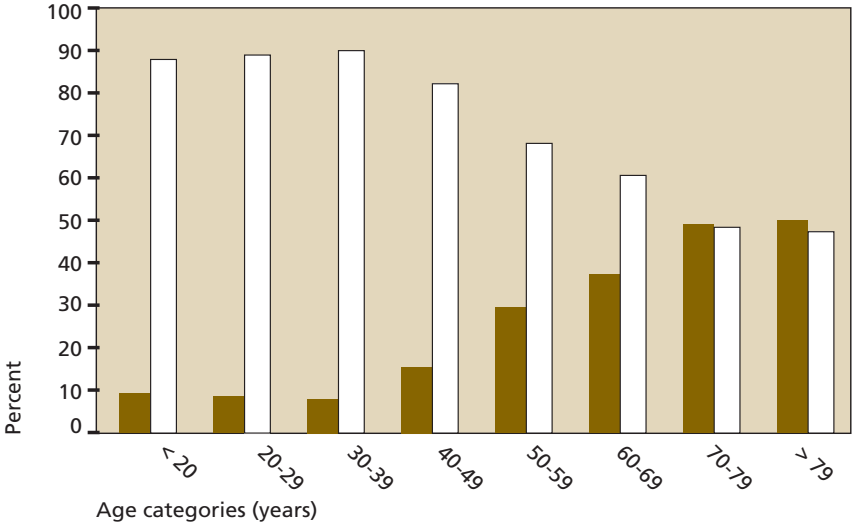

Figure 2. *Helicobacter pylori positivity in relation to age in 997 subjects from a general practice in the Netherlands (filled bars: H. pylori positive; open bars: H. pylori negative).*

H. pylori findings

In this study of 997 subjects, 272 persons (27%) were *H. pylori* positive; *H. pylori* was positive in 14% of the subjects younger than the mean age of the total group of 52 years, compared to 40% of the subjects older than 52 years. The 50% infection rate starts from the 7th age decade illustrating the cohort effect of *H. pylori* infection in the general population in the Netherlands *(Figure 2)*.

The *H. pylori* infection rate of subjects with serological atrophic corpus gastritis (62%) differed significantly from that of the whole group (27%) ($p<0.0001$). However, the *H. pylori* infection rate in the 34 subjects with serological atrophic corpus gastritis (62%) did not significantly differ from the 34 subjects in the nested case control group (50%; $p = 0.46$; *table 1*), resulting in an odds ratio (95 percent confidence interval) of 1.62 (0.62-4.24). Nevertheless, according to the well-known *H. pylori* cohort effect in Western countries, it is difficult to look for a statistical significance comparing two elderly populations (mean ages 67 and 65 years) where a high anti-*H. pylori* seroprevalence is expected.

Parietal cell antibody findings

In the whole cohort of 997 subjects, 3% had a personal or family history of autoimmune disease. In the group with serological atrophic corpus gastritis, this percentage was 6% and in the selected controls nobody had a personal or family history of autoimmune disease.

In the serological atrophic corpus gastritis group, 15 (44%) subjects had parietal cell antibodies, compared to 1 (3%) person in the nested control group ($p<0.005$, table 1), resulting in an odds ratio of 24 (95% CI, 3-201).

Eight subjects with serological atrophic corpus gastritis and anti-parietal cell antibodies were anti-*H.pylori* negative *(table 2)*. This subset of subjects had a mean age of 63 years (range 28-89), 2 of them did have other autoimmune disorders and 3 of them showed slight macrocytic anaemia.

In the atrophic corpus gastritis group 7 persons had both parietal cell and *H.pylori* antibodies. In contrast, 5 patients had *neither* parietal cell *nor H.pylori* antibodies *(table 2)*.

Intrinsic factor antibody findings

In the serological atrophic corpus gastritis group, 5 (15%) persons had intrinsic factor antibodies, compared to 2 (6%) subjects in the nested control group (n.s.).

In the atrophic gastritis group, 4 persons with antibodies to intrinsic factor had also antibodies to parietal cells.

Possible false-negative _H. pylori_ serology

Four subjects with serological atrophic corpus gastritis had an *H. pylori* absorbance index between 21-30 compared to one person in the control group. These 4 subjects had no antibodies to parietal cells. It cannot be excluded that a relatively high but

Table 1. *Characteristics and findings of 34 subjects with serological atrophic corpus gastritis compared to 34 nested case-control subjects without serological evidence of atrophic corpus gastritis.*

	Serological atrophic corpus gastritis group N=34	Nested case-control group N=34
Sex (M / F)	15 / 19	15 / 19
Age at diagnosis *Median and range*	67 (28 – 91)	65 (28 – 89)
History of complaints *Never* *Seldom* *Sometimes* *Often* *Almost always*	 59% 15% 20% 6% 0%	 56% 18% 9% 11% 6%
Gastrin (ng/l) *Median and range*	1256 (102 – 4500)	45 (18 – 108)
Pepsinogen A (_g/l) *Median and range*	6 (1 – 16)	33 (12 – 128)
Ratio PgA/C *Median and range*	0.5 (0.1 - 1.4)	2.1 (1.4 – 5.0)
Anti-H. pylori Anti- parietal cells Anti- intrinsic factor	21 of 34 (62%) 15 of 34 (44%) 5 of 34 (15%)	17 of 34 (50%) 1 of 32* _(3%)* 2 of 34 (6%)

* the serum of 2 subjects in the nested case-control group was disturbed by hetero-antibodies

* significant (P < 0.005)

Table 2. *Prevalence of anti-H.pylori and anti-parietal antibodies in 34 subjects with serological atrophic corpus gastritis.*

		Anti-*H.pylori* +ve	Anti-*H.pylori* -ve	
Anti-parietal	+ ve	7	8	15
	- ve	14	5	19
		21	13	34

+ ve = positive

- ve = negative

still normal *H. pylori* absorbance ratio between 21-30 represents persons with a recently acquired *H. pylori* infection prior to seroconversion or subjects with an infection in the past, a so-called serological *H. pylori* scar. Assuming that *H. pylori* infection in the past has contributed to atrophic gastritis in these 4 subjects, *H. pylori* may be involved in atrophic corpus gastritis in 25/34 (74%) compared to 18/34 (53%) in the nested case controls (p=0.13), resulting in a relative risk of having serological atrophic corpus gastritis for anti-*H. pylori* antibodies of 2.5 (0.9-6.8). In *table 3* the prevalence of anti-*H. pylori* and anti-PC antibodies in subjects with serological atrophic body gastritis are presented assuming that the 4 persons with *H. pylori* absorbance ratio's between 21-30 are *H. pylori* positive. Only one person with atrophic body gastritis had neither anti-*H. pylori* antibodies nor anti-PC antibodies.

Table 3. *Prevalence of anti-H.pylori antibodies adjusted for borderline H. pylori infection and anti-parietal cell antibodies in 34 subjects with serological atrophic corpus gastritis.*

		Anti-*H.p* adjusted for borderline absorbance index		
		+ve	-ve	
Anti-parietal	+ve	7	8	15
	-ve	18	1	19
		25	9	34

+ ve = positive
- ve = negative

Vitamin B_{12} deficiency
In 9 out of 25 subjects with serological atrophic corpus gastritis serum vitamin B_{12} was low (< 120 pmol/l).

History of gastric complaints
Significant differences in gastric complaints between atrophic gastritis and non-atrophic gastritis participants could not be detected: 59% of atrophic-persons, versus 56% of non-atrophic subjects never had stomach complaints; 15% versus 18% seldom; 20% versus 9% sometimes; 6% versus 11% often and 0% versus 6% had a history of almost always complaints *(table 1)*.

It is noteworthy that, analyzing the whole cohort of 997 subjects, 24% of the individuals had seldom to sometimes gastric complaints and 8% frequently. In table 1 the characteristics of the 34 persons with serological atrophic gastritis compared to their 34 nested case-control subjects are summarized.

Discussion

Our study clearly differs from all other studies on chronic atrophic gastritis because it is family practice based, carried out in a large unselected primary cohort and it uses a nested case-control group without serological atrophy. A limitation of secondary care studies and studies with volunteers (8) on the subject of chronic atrophic gastritis is the use of a highly selected group of patients, often with clinical disease. We enrolled consecutive subjects visiting a general practice for common health questions. The sample is self selected by attendance at the GP's surgery but the study population can be considered as a reflection of the general population [31]. In our consecutive sample of 997 general practice patients, 34 (3.4%) individuals were identified using serological markers, as having atrophic corpus gastritis. It was shown that the prevalence of *H. pylori* infection (62%) in subjects with serological atrophic corpus gastritis in a primary care community in Western Europe did not appear to be significantly higher compared to a nested control group without serological atrophic corpus gastritis (50%). On the contrary, the prevalence of antibodies to parietal cells, as a manifestation of autoimmunity, was significantly higher (44%) in the serological atrophy group than in the nested control group (3%). The odds ratio of having serological atrophic corpus gastritis was much higher ($p < 0.025$) for parietal cell antibodies (24) than for *H. pylori* antibodies (1.62). It is therefore attractive to pose that, apart from *H. pylori*, autoimmunity is involved in the evolution of atrophic corpus gastritis in this part of the Netherlands. About the impact of *H. pylori*, it is interesting to note that an additional 4 subjects with seroatrophy showed anti-*H. pylori* IgG-levels just below the serological cut-off point, suggesting previous extinguished *H. pylori* infection. Inclusion of these 4 subjects augments the overall prevalence of *H. pylori* to 72%, a percentage which approaches the *H. pylori* prevalence in atrophic corpus gastritis found by Annibale et al (32). It is also noteworthy that 2 young female subjects with serological atrophic corpus gastritis, aged 28 and 42 years, respectively, had no antibodies to *H. pylori* but antibodies to parietal cells. At a young age it is very improbable that *H. pylori* antibodies have already been extinguished.

A study by Bins et al. in 1984 in another part of the Netherlands, investigating the prevalence of pentagastrin refractory achlorhydria in a general population by gastric intubation, revealed a prevalence of 2.4% (33). The difference with a prevalence of 3.4% severe atrophic corpus gastritis in our study might be due to the difference in the age of the study population. Bins et al. studied subjects and their spouses working in a factory resulting in a maximum age of about 65 years. In our study 24 of the 34 subjects with serological atrophic gastritis were over 65 years.

Acquired infection with *H. pylori* in early childhood (34-36) and a host response in developing autoimmune reactions against organ specific tissue like the gastric mucosa are both aetiopathogenic factors in the evolution of atrophic gastritis

and subsequently via intestinal metaplasia and dysplasia with progression to gastric cancer [1,37].

These aetiopathogenetic factors may act separately but they may also influence each other leading to increased progression of atrophic changes. This increased progression to gastric atrophy may result in a decrease of the *H. pylori* infection rate shown in a gastric biopsy study [38]. Although anti-*H. pylori* antibodies remain positive for many years after *H. pylori* eradication from the gastric mucosa, it is not possible to exclude with certainty that the *H. pylori*-negative subjects in our study had been infected by *H. pylori* long ago [39].

Our 7 atrophy subjects with both *H. pylori* and parietal cell antibodies illustrate their possible concomitant and/or sequential part in the progression of atrophic corpus gastritis [18,38]. It has been suggested that antibodies to parietal cells are secondary to the tissue damage induced by the *H. pylori* infection (40-42). However, 8 of the subjects with parietal cell antibodies had no evidence of previous *H. pylori* infection, 2 of this group being quite young, aged 28 and 42 years.

As mentioned above, in 4 of the 5 atrophy subjects with negative serological antibody reaction to both *H. pylori* and parietal cells, the IgG absorbance index for *H. pylori* was just below the upper limit of normal, suggesting the possibility that the immune response to *H. pylori* is extinguishing. Because decades may pass between initiation and detection of gastric atrophy and the atrophic microenvironment promotes spontaneous eradication [39], substantial misclassification of relevant exposure to *H. pylori* may occur. This misclassification due to fading *H. pylori* infection is much more relevant for studies using gastric biopsies than for studies using *H. pylori* serology. It is noteworthy that we used a type of enzyme immunoassay known to be most succesful for documentation of previous *H. pylori* infection [26,43].

In a review of the medication history of all the *H. pylori* negative subjects there was no evidence of use of antibiotics during the 4 years before blood sampling.

This study indicates that most patients with serological atrophic corpus gastritis did not present more frequently with a history of gastric complaints than did control subjects. Thus early detection of atrophic gastritis is not possible on account of complaints [7]. About 35% of patients with serological atrophic corpus gastritis in our study had low vitamin B_{12} serum levels as a silent clinical condition. Cobalamin supplementation is now possible.

This explorative study has the advantage that the population studied is enrolled in one general practice and that the research is supervised by the primary care physician himself who knows everybody personally and is familiar with the family roots of his patients. Furthermore, this general practitioner happens to be also pharmacist for his own patients, so the use of drugs is accurately documented and controlled.

In *conclusion,* the seroprevalence of atrophic corpus gastritis, in a Dutch primary care community is 3.4%. This study further shows that in the indigenous West-European population serological atrophic corpus gastritis is due both to *H. py-*

lori infection and gastric autoimmunity. In our study at the primary care level serological atrophic corpus gastritis was significantly associated with gastric autoimmunity with an odds ratio of 24.0, while a non-significant odds ratio of 1.62 was found with H.pylori infection. In view of the decreasing risk of *H. pylori* infection in the Western world it is likely that the impact of *H. pylori* on the development of atrophic corpus gastritis will further diminish.

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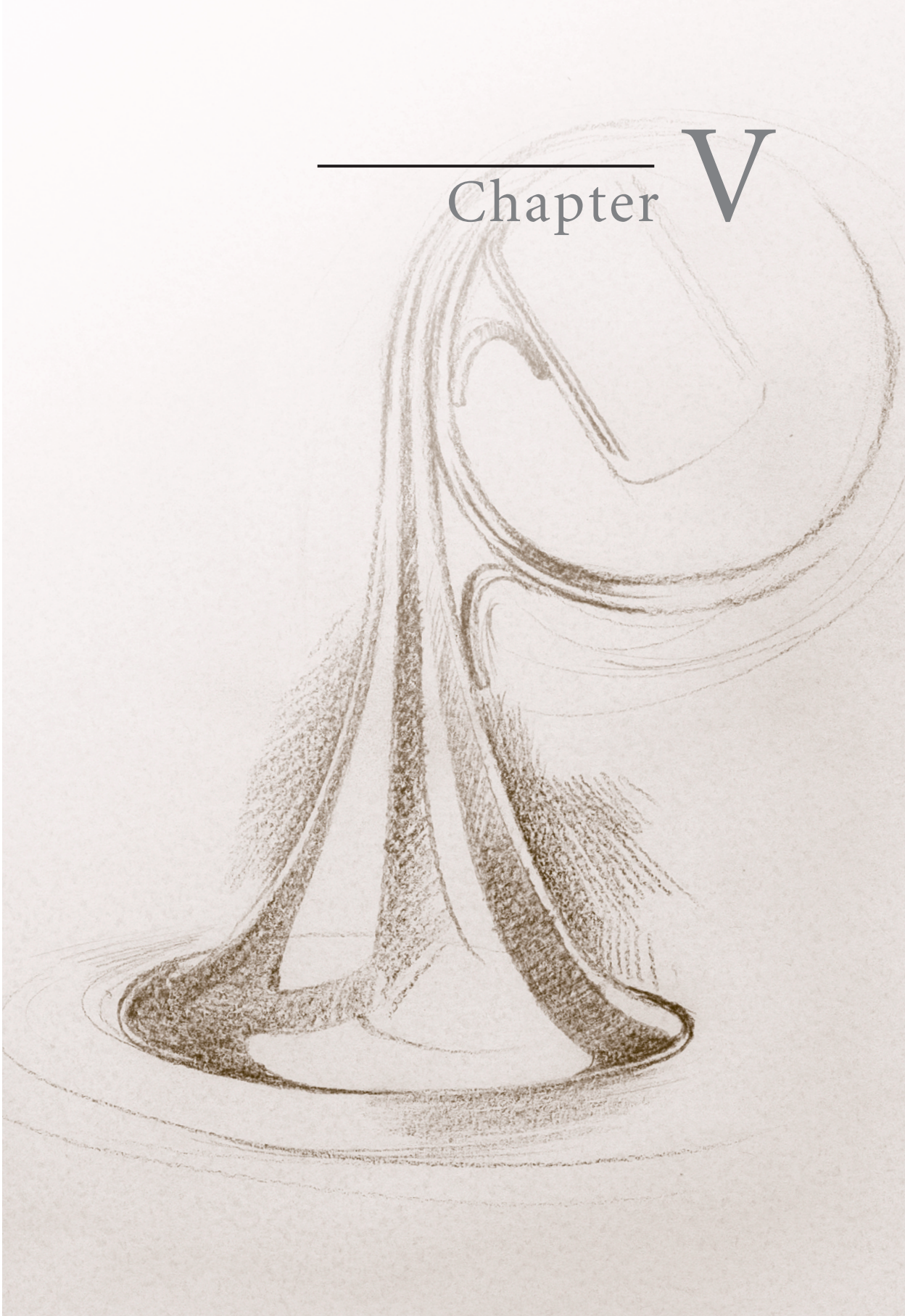
Chapter V



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Medicine is a lifelong education.

(Sir William Osler, 1848-1920)

◆

Chapter V

Comparison between serology, endoscopy and histology in the diagnosis of advanced gastric body atrophy: a study in a Dutch primary care community

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Keywords: gastric body atrophy, serum biopsy, pepsinogen, gastrin, *Helicobacter pylori*, vitamine B_{12}, primary care.

Abstract

Background: Gastric body atrophy (GBA) is a precursor lesion of gastric cancer. Little is known about the value of serological screening for GBA in a primary health care community.

Aim: To study the relation between serological findings, endoscopical and histological GBA changes in a sample of the general population.

Subjects & methods: Consecutive adults (n = 997) were serologically screened for GBA in a Dutch family practice. Thirtyfour subjects had serological GBA defined as hypergastrinaemia (> 100 ng/l), hypopepsinogenaemia A (< 17 µg/l) and a low pepsinogen A/C ratio (<1.6). Twentyfive subjects of this group, agreed in serological retesting and further investigation with gastroscopy and biopsy for assessment according to the Sydney system.

Results: At serological retesting 20 of 25 subjects again fulfilled the criteria of GBA. Histological examination of the corpus biopsies showed advanced GBA in 18 of 24 subjects (75%,1 subject had no corpus biopsies) and 17 of 19 (89%) subjects with repeated positive serology. After disclosure of serology results, re-examination of the biopsies revealed GBA also in the 2 patients with initially insufficient

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evidence of GBA, giving a concordance of 100% [19/19]. One subject with normal serum gastrin at retesting, thus no longer fulfilling the serological criteria of GBA (false-negative), had both antral and body atrophy giving a concordance between serological and histological GBA of 95% [19/20]. No adenomatous polyps, tumours or dysplastic alterations were found. Macroscopic features observed during gastroscopy were of no value in the assessment of atrophy.

Conclusions: Identification by serology of subjects with chronic atrophic body gastritis in population-based screening and prevention studies in primary care is adequately possible and useful in selecting subjects for endoscopy.

Introduction

Chronic atrophic gastritis is a chronic inflammation of the stomach accompanied by loss of the specialized glandular cells [1]. Atrophy leads to thinning of the mucosa and is a common denominator in all pathological processes, causing severe mucosal damage. The development of chronic atrophic gastritis is a multifactorial process, involving microbiological factors like *Helicobacter pylori* infection, unidentified host and environmental factors, or autoimmunity directed against gastric glandular cells [1-4]. The autoimmune form of gastritis is typically located in the body, whereas the type induced by chronic injury or infection is located more often in the antrum. The loss of the glandular structures can be accompanied by metaplasia.

A large number of studies point to the importance of chronic gastritis in the evolution of such gastritis towards mucosal atrophy, intestinal metaplasia, dysplasia and finally gastric adenocarcinoma [5-7]. Early identification of patients with atrophic gastritis might give opportunities in modifying the risk of gastric cancer, which is nowadays the second leading cause of cancer-related mortality worldwide, having a very poor clinical prognosis (8). Histological examination is the most reliable way to determine atrophic gastritis, but this is not done routinely in patients without gastric cancer. Its principal use is to rule out the presence of cancer rather than to determine the presence and extent of atrophic gastritis. The by far preferable diagnostic instrument to screen for atrophic gastritis is a serum biopsy, i.e. measuring serum pepsinogen A and C and serum gastrin as functional markers of the gastric mucosa [9,10]. Low serum pepsinogen A (PgA) and a low pepsinogen A/C ratio in combination with elevated serum gastrin, are considered useful predictors of gastric body atrophy (GBA). Several studies have shown the diagnostic potential of non-invasive, serological biomarkers for atrophic gastritis [11-14]. Taking into account the underlying aetiology of chronic atrophic gastritis, serological testing for *H. pylori* [2-4,10] and parietal cell antibodies has an additional diagnostic value [10,14].

Apart from predisposing to gastric cancer, gastric body atrophy can also lead to vitamin B_{12}-deficiency. This occurs with both autoimmune corpus gastritis and

the more common multifocal pangastritis involving corpus and antrum that results from *H. pylori* infection. This can exist already for a long time as a clinical latent entity with possible irreversible cell damage to the nervous system [5,15]. Early detection of vitamine B_{12}-deficiency is therefore important and timely supplementation is indicated to prevent pernicious anaemia and neurological sequelae. It is likely that a significant number of patients suffering from a deficiency of vitamin B^{12}caused by atrophic gastritis remain undiagnosed and untreated and that, with regard to public health, it would be important to identify these patients early enough [16].

Despite the apparent importance of the diagnostic potential of serological methods, no studies have been conducted in general practice, so far, to determine the significance of serology in the screening for atrophic body gastritis. The purpose of the present study was to evaluate the value of the serological markers of atrophy, i.e. blood pepsinogen and gastrin levels, to predict histological GBA in a community-based family practice in The Netherlands. Additionally, attention was payed to the prevalence of *H. pylori* infection on the basis of serological tests and histological examination and to the prevalence of autoimmunity in gastric atrophy.

Subjects and methods

Study Population

In a period of 2 years a total of 997 adults, consecutively entering the primary health-care system because of common medical problems, volunteered in serological screening for GBA in the general practice in 's-Gravenpolder, a rural village in the South-West of The Netherlands. Exclusion criteria were age < 18 years, current pregnancy, gastric resection, renal insufficiency and the use of antisecretory agents. The participants were asked to donate a fasting blood sample and to fill in a questionnaire describing the frequency and severity of gastric symptoms during the preceding 3-month period, past gastric diseases and the use of stomach- and/or antibiotic drugs.

Examination of the whole group revealed serological GBA in 34 persons (3.4%, 15 M, 19 F; mean age 67 years, range 28-91).

Two years later, 25 of the 34 subjects (12 M, 13 F; mean age 67 years) agreed in undergoing upper gastrointestinal endoscopy after an overnight fast, combined with serological retesting of the markers of atrophy and *H. pylori*- and autoimmune serology. The remaining 9 subjects were not biopsied for the following reasons: 3 persons died of cardiovascular related diseases, 2 persons had moved out of the region, 2 persons were not able to undergo endoscopy because of serious not gastro-intestinal related comorbidity and 2 persons refused further investigation.

The study was performed according to the declaration of Helsinki and all participants gave informed consent before entering the study.

Serological examination
All obtained serum samples were tested by well-validated radio-immunoassays for levels of pepsinogen A (PgA), pepsinogen C (PgC) and gastrin [17]. Our validated criteria for advanced serological GBA, corresponding to pentagastrine refractory achlorhydria or severe hypochlorhydria (peak acid output < 5 mmol/hr), expressed in the level of the serum markers, were a serum concentration of PgA < 17 μg/l, a PgA/C ratio < 1.6 and an accompanying serum concentration of gastrin > 100 ng/l[17]. *H. pylori* serology was performed by a validated enzyme immunoassay using specific immunoglobulin G against *H. pylori*. The results were expressed as the absorbance index (AI): serum with an AI > 0.32 IgG *H. pylori* antibody was considered evidence of *H. pylori* infection [18]. Additionally, all serum samples were tested for parietal cell- and intrinsic factor autoantibodies using commercially available kits, respectively Autoscreen 1, Scimedx Corporation, Denville, NJ 07834, USA and Genesis Diagnostics Ltd, Little port, UK. Serum vitamine B_{12} concentration was tested by Immulite ®2000 Vitamin B^{12}, Diagnostic Products Corporation, Los Angeles, CA 90045-5597, USA.

Endoscopic Examination
Patients fasted for at least 9 hours before the examination. Gastroscopy was performed in the usual manner using an Olympus video-endoscopy equipment. Endoscopic characteristics and appearances of gastric mucosal inflammation were recorded for each subject according to the Sydney System, Endoscopic division [19]. The following characteristics were scored: normal mucosa with its pink colour, with uniform smoothness and lustre, versus endoscopic characteristics of inflammation e.g. 1. edema - 2. erythema - 3. friability - 4. exudates - 5. flat erosions - 6. raised erosions - 7. rugal hyperplasia - 8. rugal atrophy - 9. visibility of vascular pattern - 10. intramural bleeding spots - 11. nodularity. After maximum air insufflation at the end of examination, the corpus was examined for the presence of rugae. The grade of the various macroscopic features was scored as absent, mild, moderate or severe.

Biopsy Collection
Biopsy samples were obtained using a standard pinch-biopsy forceps. Antral and fundic biopsy specimens were systematically collected as follows: 6 biopsies from the mid antrum , about 2 cm pre-pyloric from the anterior and posterior antral wall, 4 for histological examination, 2 for culture; 6 biopsies from the mid body, about 5 cm distal of the oesophagus-cardia junction from the anterior and posterior body wall, also 4 for histological examination and 2 for culture. Biopsies for histology were fixed in 10% buffered formalin.

Histological Examination
All biopsies from each subject were routinely fixed in 10% buffered formalin and em-

bedded in paraffin blocks. Five micron sections were hematoxylin and eosin stained and examined by 2 expert gastrointestinal pathologists (SvE & GJAO) according to the updated Sydney classification. Additional immunostaining was performed with antibodies against gastrin, chromogranin and *H. pylori* to identify gastrin producing G-cells, enterochromaffin-like cells and *H. pylori*, respectively. The diagnosis of chronic atrophic gastritis was based on the full spectrum of the updated Sydney System scores [1,19], i.e. chronic inflammation, activity, glandular atrophy, intestinal metaplasia and absence or presence of *H. pylori*, systematically applied to the biopsy specimens. Chronic inflammation was evaluated on the basis of an increase of mononuclear cell infiltration of the lamina propria. Activity of gastritis was defined according to the presence or absence of intra-epithelial granulocytes. Gastric atrophy refers to the loss of the deeper specialized glands, i.e. in the body the parietal cells and secondly the chief cells. The loss of the glandular structures can be accompanied by metaplasia [1]. All histopathological issues were semi-quantitatively graded as absent, mild, moderate or severe. The biopsy specimens were reviewed by the 2 above mentioned experienced pathologists. In case of disagreement the pathologists discussed the case to reach consensus.

Results

Retesting of serum markers of atrophy

Re-testing of the markers of atrophy, undertaken 2 years after the initial screening and immediately before endoscopy, revealed serological gastric body atrophy only in 20 subjects of the whole group of 25 *(Table 1)*.

The 5 "drop-out" subjects showed the following serological characteristics: 1 subject (no. 8) had after retesting normal gastrin with low PgA and low ratio PgA/C, thus no longer fulfilling the serological criteria of GBA. The remaining 4 subjects showed after retesting normal serum pepsinogens and normal gastrin, of whom 2 persons (nos. 3 and 14) had borderline test results in the first round and the other 2 individuals (nos. 6 and 15) had an unexplained conversion to normal levels of the serum markers *(Table 1)*.

Histopathologic findings *(see Table 1)*

As expected after serologically retesting, 4 individuals with normal serology (nos. 3,6,14,15) had no evidence of histological body atrophy, but only modest aspecific chronic inflammation. One subject (no. 8), with normal gastrin and low PgA with a low A/C ratio, thus partially fulfilling the criteria of serological body atrophy, had antral and body atrophy in the biopsies, so making the serological profile false negative.

Moderate to severe body atrophy was found in 17 of 20 subjects with repeated serological GBA. With regard to the other 3 subjects: from 1 subject (no. 4) only

Table 1. *Serological, endoscopical and histological characteristics in 25 primary healthcare subjects with focus on gastric atrophy*

	Serological markers			*Serology after 2 yrs*			*Serology*	*Histology*		*Endoscopy*
Nr	Gastrin	PgA	PgA/C ratio	Gastrin retest	PgA retest	PgA/C ratio retest	GBA after retest	GBA after retest	GAA after retest	GBA after retest
1	1760	2	0.3	1664	1	0.3	+	+++	—	—
2	1245	2	0.1	1269	2	0.2	+	+++	—	—
3	102	12	1.4	86	17	2.7	—	—	—	—
4	3100	2	0.3	2212	1	0.3	+	□ *	—	+
5	239	3	0.3	123	3	0.4	+	+++	+	+
6	193	9	0.5	134	25	1.7	—	—	—	—
7	2885	7	0.6	933	8	0.9	+	+++	—	—
8	184	16	0.6	68	16	0.7	—	+	+	—
9	1995	1	0.1	764	1	0.1	+	+++	—	—
10	1750	5	0.4	534	2	0.2	+	+++	—	+
11	2300	2	0.1	2086	0	0.1	+	+++	—	—
12	1960	2	0.3	1352	1	0.4	+	+++	—	+
13	1391	13	0.6	1791	3	0.3	+	++	—	+
14	156	14	1.2	59	17	3.5	—	—	—	+
15	940	7	0.4	77	9	5.3	—	—	○ *	—
16	137	5	0.4	116	3	0.7	+	+++	+++	—
17	324	7	0.3	628	9	0.8	+	+++	△ *	—
18	593	3	0.3	552	3	0.7	+	++	—	+
19	486	7	0.3	459	5	0.3	+	++	+	—
20	418	2	0.5	146	6	1.3	+	++	—	—
21	1775	6	0.7	2257	1	0.3	+	+++	—	—
22	492	15	0.8	1179	14	0.8	+	++	+	—
23	259	2	0.1	159	4	0.3	+	++	++	—
24	488	4	0.3	1284	6	0.5	+	+++	++	+
25	438	7	1.0	284	11	0.8	+	+++	+++	—

□ * no corpus biopsy specimens
○ * no antrum biopsy specimens
△ * too small biopsy specimens, no optimal diagnosis
PgA = pepsinogen A
PgA/C ratio = ratio pepsinogen A / pepsinogen C
GBA = gastric body atrophy
GAA = gastric antral atrophy
– = absent, + = mild, ++ = moderate, +++ = severe

Table 2. *Anti-H. pylori and autoimmune serology in 20 histological gastric body atrophy subjects*

		Anti-PC & IF		
		+ve	– ve	Total
Anti-*H. pylori*	+ ve	4	8	12
	– ve	7	1	8
Total		11	9	20

Table 3. *Anti-H. pylori and autoimmunity in 8 subjects with vitamine B_{12} deficiency*

		Anti-PC & IF		
		+ve	– ve	Total
Anti-*H. pylori*	+ ve	2	1	3
	– ve	4	1	5
Total		6	2	8

Anti-H.pylori = antibodies to Helicobacter pylori
Anti-PC = antibodies to parietal cells
Anti IF = antibodies to intrinsic factor
+ ve = positive
– ve = negative

Table 4. *Characteristics and functional findings at diagnosis of 20 subjects with histological gastric body atrophy*

Sex (M / F)	11 / 9	
Median age at diagnosis (yrs)	68	range (31-93)
History of complaints		
Never	11 subjects	(55%)
Seldom	4	(20%)
Sometimes	3	(15%)
Often	2	(10%)
Positive *H. pylori* serology	12	(60%)
Positive *H. pylori* histology	7	(35%)
Autoimmunity (antibodies-parietal cells & intrinsic factor detectable)	11	(55%)
H. pylori & autoimmunity positive	4	(20%)
Vitamine B^{12} level ↓ (<120 pmol/l)	8	(40%) (range 53-399)
Median serum gastrin level(ng/l)	882	*range* (68 – 2257)
Median pepsinogen A level (μg/l)	5	*range* (0 – 14)

antrum biopsy specimens and no corpus biopsies were available; the second person (no. 23) had severe *H. pylori* gastritis, preventing optimal grading of atrophic changes and the third subject (no. 17) had aspecific inflammatory cell infiltration with atrophy that was difficult to recognize.

Because of the discrepancy between serological and histological results in the 2 subjects and the retest "serum biopsy" showing a repeat outspoken atrophy profile, restudy of histology showed the presence of body atrophy after all, also in these 2 serologically atrophic subjects (nos. 17 and 23).

Antral biopsies showed atrophic changes in 8 of 23 subjects. One subject had no antrum biopsies (no.15), another subject had inadequate antral biopsies for optimal histodiagnostical investigation (no.17). *H. pylori* was identified in the biopsies of 7 (35%) of 20 persons with GBA. Eleven GBA subjects showed evidence of ECL-cell hyperplasia in the corpus biopsy specimens. None of the investigated subjects showed evidence of gastric dysplasia or malignancy.

Serological results related to histological findings

On the basis of the serologic findings after retesting and histological reviewing, all 19 persons showed histological GBA. One histological GBA-subject with a normal serum gastrin at retesting, therefore only partially fulfilling the serological GBA criteria (false-negative), had developed antral intestinal metaplasia in addition to GBA (no. 8 in table 1), giving a concordance between serological and histological GBA of 95% [19/20].

Endoscopical diagnoses

In 8 subjects (40%) of the group of 20 individuals with repeated serological GBA , moderate to severe atrophic gastritis of the corpus was diagnosed endoscopically, based on the presence of a vascular pattern in a slightly distended stomach with absent or flattened folds. In 2 of those 8 persons also atrophic gastritis of the antrum was found endoscopically. Greyish patches, histologically corresponding with intestinal metaplasia, were found in 2 of the 8 subjects. In 10 of the remaining 12 subjects, an erythematous exudative gastritis was diagnosed. Finally, 2 subjects had a flat erosive gastritis.

The 5 "drop-out"subjects without serological body atrophy showed the following endoscopical appearances: person nos. 3, 6 and 8 erythematous exudative, no. 14 mild atrophic and no 15 flat erosive.

Endoscopical features related to histological findings

The endoscopical diagnosis of body atrophy, made in 8 subjects of 20, was confirmed histologically in 6 of them. One subject had no corpus biopsies and the other person (nr. 14) had only modest chronic inflammation in the corpus biopsies. The absence of folds and presence of visible vessels, as gastroscopic atrophy features, had only a positive predictive value in the diagnosis of body atrophy, but the absence or presence of visible vessels had no diagnostic value in antrum atrophy.

In 4 persons with normal endoscopical features the biopsies showed mild to severe corpus atrophy and a normal to mild antrum atrophy.

The 11 subjects with macroscopic erythematous exudative gastritis had the following histological diagnosis: 6 showed moderate to severe body atrophy and 5 had a normal histological appearance. The remaining 2 individuals with macroscopic flat erosive gastritis showed advanced body atrophy and a modest chronic body gastritis, respectively. Biopsies of greyish patches confirmed the endoscopical diagnosis of intestinal metaplasia.

Overall, there appeared to be a very weak correlation between endoscopical and histological findings.

H. pylori- and autoimmune serology in GBA-patients

H. pylori serology was positive in 12 (60%) of the 20 histologically identified GBA persons. Eleven (55%) of 20 GBA subjects had antibodies to parietal cells and/or antibodies to intrinsic factor (1 person had only antibodies to intrinsic factor); 4 persons had both antibodies to *H. pylori* and to parietal cells (20%). One female person was seronegative to *H. pylori* and parietal cells and intrinsic factor. She appeared to have an IgG AI 0.29, so near the serological cut off point, indicating an extinguished *H. pylori* infection *(Table 2)*.

After retesting it is worth noting that a *H. pylori*-seroconversion of 2 subjects (nos. 14 and 25) had taken place in the elapsed 2 years, respectively from *H. pylori* seropositive to negative and the reverse way in the other patient. Patient no. 14 showed also the abovementioned conversion from serological body atrophy to a normal profile.

Vitamine B_{12} deficiency

The levels of all histological GBA persons ranged from 53 to 399 pmol/l.

Eight (40%) subjects of 20 histological GBA persons had low (< 120 pmol/l) serum vitamine B_{12} levels. The distribution of *H. pylori* and autoimmunity in our GBA-subjects with vitamine B_{12}-deficiency is shown in *table 3*.

History of gastric complaints

Analysis of the questionnaires of the 20 subjects with histological GBA on the frequency and severity of any epigastric pain, heartburn, nausea, belching and acid regurgitation, showed that 11 persons (55%) never had stomach complaints, 4 (20%) seldom, 3 (15%) sometimes and 2 persons (10%) often. None of the subjects had gastric complaints severe enough to consult a doctor or to use drugs.

Of the 4 individuals with a normal serological profile after retesting and without histological body atrophy, 2 had never and 2 had sometimes gastric complaints.

Finally, the characteristics and functional findings of the 20 subjects with GBA are summarized in *table 4.*

Discussion

The value of serology for the histological diagnosis of gastric body atrophy was assessed in a Dutch general practice. To our best knowledge, this is the first study in which the diagnostic performance of the gastric serum biopsy is investigated in a primary health care setting.

Although serological gastric investigation is already more than 2 decades generally accepted as a diagnostic non-invasive tool [20], it has never got appropriate attention in primary care.

Most of the data currently available, regarding the prevalence of chronic atrophic gastritis in the general population, are obtained from endoscopical studies of hospital outpatients with dyspeptic problems, referred to Gastroenterology Departments of hospitals [9,21]. The additional value of our study is that we enrolled a large number (997) of adult subjects, consecutively entering the general practice for common health questions, so we were able to screen a representative sample of the general indigenous Dutch population.

In the present study the result of serological retesting, 2 years after the initial serological screening, is noteworthy. In the evaluable group of 25 individuals for endoscopy, only 20 subjects appeared to be sero-atrophic at retesting. Retesting is advisible in cases of borderline test results and when a substantial interval in time elapses between screening and follow up investigation. Serological results are most reliable immediately before endoscopy because of certainty that the target group of subjects has an empty stomach. Moreover, because the serological and histological biopsy are taken at the same time, the mutual comparison is more reliable. The serum biopsy is a biologic parameter and consequently liable to variability. Taking into account the dynamics of gastritis, progress (patient nr. 8) and also regress (patient nr.14) of atrophic gastritis can be reported.

Considering the histological examination after serological retesting, all 19 subjects with serological GBA and corpus biopsies, showed histological GBA, giving a concordance of 100%. However, 1 subject with normal serum gastrin at retesting, therefore only partially fulfilling the serological atrophy criteria (false-negative), had antral atrophy with intestinal metaplasia in addition to GBA (patient nr. 8), giving a concordance between serological and histological GBA of 95% [19/20]. In fact this was the single subject among 20 individuals with GBA who had serological evidence of antral atrophy. The rarity of serological antral atrophy in subjects with GBA is in agreement with a study by Bins et al. in factory workers and their spouses, who reported a single case with normal gastrin pointing to antral atrophy in one of 14 subjects with pentagastrin-refractory achlorhydria [22].

Furthermore, our study confirms that macroscopic diagnoses with regard to atrophy as observed during endoscopy are of limited value. Only 6 of 20 patients with gastric atrophy were identified. The remaining 14 atrophy-patients had variable diagnoses, mainly erythematous exudative gastritis but also flat erosive gastritis

and even a normal appearance. Except for the absence of rugae and visible vessels in the fully insufflated stomach, as signs of moderate to severe atrophic corpus gastritis, macroscopic features as observed during gastroscopy appeared to have a very weak correlation with histological findings. This is in accordance with most previous studies in patient populations and it must be emphasized that the diagnosis of atrophic gastritis should be based on serological and histological examination of the gastric mucosa [19,21,23].

Gastric atrophy is the endpoint of chronic active gastritis, mainly caused by *H. pylori* infection [3,4,24] or gastric autoimmunity associated with pernicious anaemia [25]. Therefore in our GBA group we investigated the prevalence of *H. pylori* infection and autoantibodies to parietal cells and intrinsic factor.

It is reported that PgA is up-regulated in *H. pylori* infected patients compared with *H. pylori*- uninfected patients. Consequently, the serological finding of a low serum PgA level and a low PgA/C ratio in a *H. pylori* infected patient is strongly indicative of gastric body atrophy [26].

H. pylori was identified histologically, using hematoxylin and eosin and immunohistochemical stains, in 7 (35%) of the 20 individuals with GBA while *H. pylori* serology was positive in 12 (60%) subjects. Routine biopsy sampling may underestimate the true prevalence of *H. pylori* infection in diffuse atrophic gastritis [27]. Therefore serological testing of *H. pylori* infection in gastric atrophy is warranted in such patients [28]. Apart from that, considering the natural course of *H. pylori* antibodies and gastric mucosal histology in patients with advanced atrophic gastritis, *H. pylori* antibodies disappear spontaneously within 10 years in almost one fourth of patients with advanced atrophic corpus gastritis. The disappearance of *H. pylori* antibodies is accompanied by no or only a mild improvement of the gastric mucosa [29].

The conversion to seronegativity found in 1 subject in this study may belong to the category of spontaneous disappearance of *H. pylori* antibodies and the conversion to seropositivity in another person may point to a denovo infection or recrudescence.

Regarding the autoimmune pathogenesis of gastric body atrophy, in this primary care study 11 (55%) of 20 persons with serological atrophy at retesting had antibodies to parietal cells and/or antibodies to intrinsic factor.

In the current study all 20 patients with histological proven atrophy had either antibodies to *H. pylori* (60%) or to parietal cell and intrinsic factor (55%), which is in agreement with the etiology of gastric atrophy. Four (20%) patients had both *H. pylori* and autoimmune antibodies which finding matches with several studies on a possible role of *H. pylori* in the development of autoimmune gastritis [30-32]. One patient was overall seronegative but had an IgG AI to *H. pylori* of 0.29, near below the serological upper limit, pointing to *H. pylori* infection in the past, a so-called serological scar.

According to the histological diagnosis of gastric body atrophy, our study revealed vitamine B_{12}-deficiency in several participants: 8 (40%) subjects of the group of 20 GBA patients showed a low serum vitamin B_{12}-levels under the reference values, 7 of them were previously unknown with a deficiency. Adequate supplementation has been started by now. In the causal distribution in our study population autoimmunity dominates over *H. pylori (table 3)*.

Because of the evident hypergastrinaemia in advanced GBA, attention was also payed to the prevalence of histamine secreting enterochromaffin-like cell (ECL) hyperplasia in the corpus biopsies. Gastrin has a trophic effect on the ECL cells of the oxyntic mucosa, stimulating their function and proliferation [33]. ECL-cell hyperplasia in the gastric corpus is a common feature in diffuse chronic atrophic gastritis restricted to the fundus, with or without associated pernicious anaemia and in *H. pylori*-related multifocal chronic atrophic gastritis [34]. In our study 11 of the investigated subjects with GBA had ECL-cell hyperplasia. There was no evidence of development from hyperplastic ECL cells into carcinoid tumors.

This study in a Dutch general community confirms previously reported data showing that a high serum gastrin and a low serum pepsinogen A, together with a low pepsinogen A/C ratio, are good predictors of the presence of atrophic body gastritis as a cancer-prone lesion. A reliable serological test to detect GBA is important for population-based screening and prevention studies. The serological gastric biopsy has the advantage that it reflects the status of the whole stomach. However, histological investigation remains always essential for the ultimate management of gastric diseases and is the reference standard in the detection of atrophic gastritis. Actually, serological and histological methods may be complementary to each other in the diagnostic assessment of atrophic gastric pathology. In the absence of widespread screening recommendations in primary care, the early detection and prevention of gastric cancer will depend on individual forward-thinking practitioners. In recent years, much attention and research efforts have emerged to identify high risk groups. As such, compelling evidence now indicates that first-degree relatives of patients with gastric cancer carry an increased risk of developing atrophic body gastritis in the presence of *H. pylori* infection [7,35].

Finally, our study confirms that there is no relation between atrophy and gastric complaints. None of the subjects with GBA had gastric complaints severe enough to consult a doctor or to use drugs. So, the only way to detect subjects with gastric atrophy, with focus on people at risk, is screening with a serum biopsy.

This study was performed in a group of subjects, selected from a large cohort on the basis of serological evidence of GBA. It can however not be excluded that there are subjects with histological GBA among the subjects who did not fulfill the serological criteria of GBA.

Anyhow, we feel that endoscopy of subjects who have fully negative serology is ethically unacceptable. The 95% concordance between serological and histologi-

cal GBA in our study does suggest that the prevalence of "unsuspected" atrophy seems negligible low.

In *conclusion*, this study in a community-based family practice emphasizes the diagnostic value of gastric serology for general practitioners. A serological gastric biopsy can be used as a reliable non-invasive screening instrument in basic health care centers in selecting patients for further invasive investigation for chronic atrophic body gastritis and should be recommended in the assessment of subjects at risk for gastric cancer.

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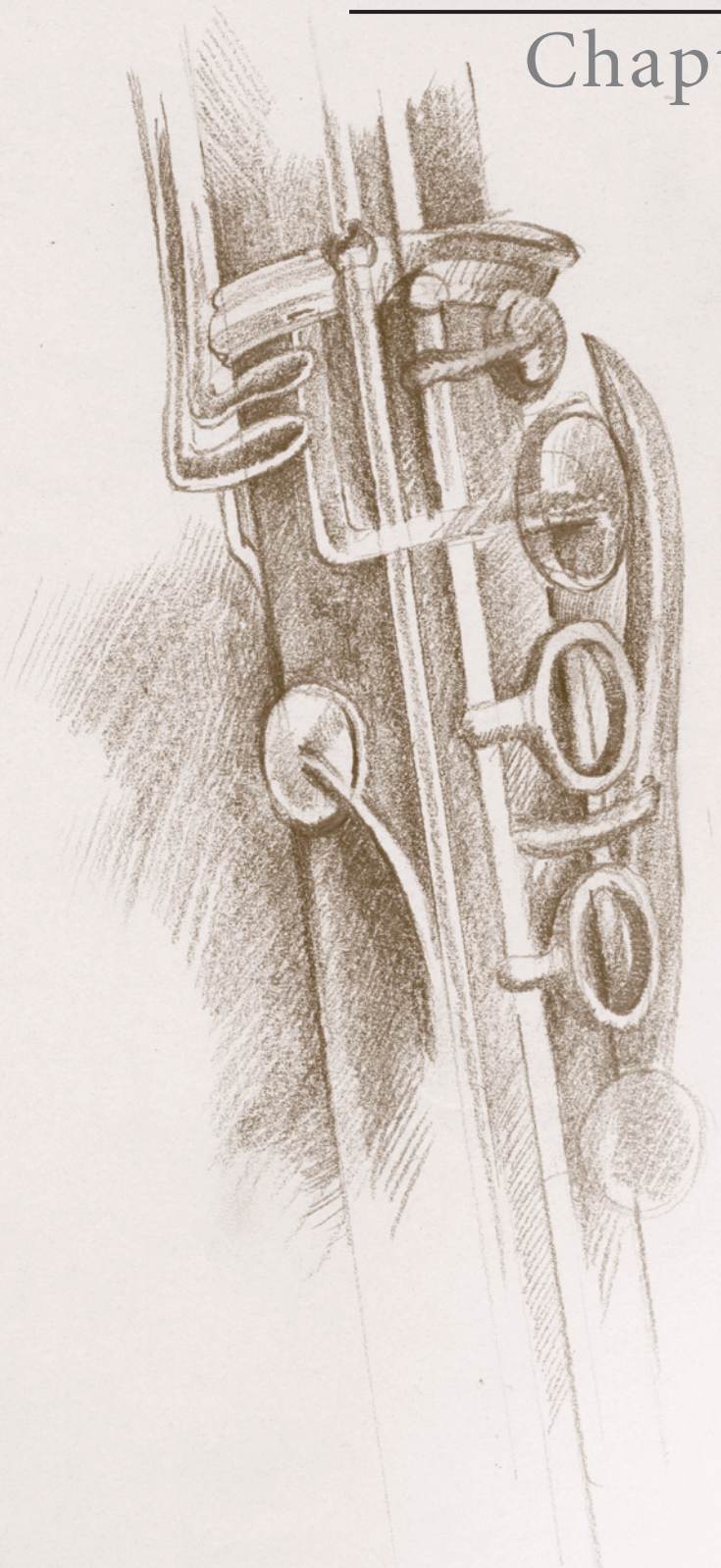
Chapter VI



◆

Wellness cannot be measured,
yet we seek it with analytic methods.

◆

Chapter VI

The ^{13}Carbon urea breath test for the diagnosis of *Helicobacter pylori* infection in subjects with atrophic gastritis: evaluation in a primary care setting

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Keywords: urea breath test, atrophic gastritis, *Helicobacter pylori*, primary care

Abstract

Background: *Helicobacter pylori* infection is the main cause of atrophic body gastritis, which may progress over time into the atrophic form, a disorder at increased risk for gastric cancer. Therefore this condition requires timely intervention by eradication of *H. pylori*. Serological detection of antibodies to *H. pylori* shows both present and past infection. The non-invasive ^{13}C-UBT is an attractive *H. pylori* diagnostic test because it detects current infection. The diagnostic value of ^{13}C-UBT has been reported to be of limited value in selected patients with atrophic body gastritis or in persons using acid lowering medication.

Aim: To determine the accuracy of ^{13}C-UBT for *H. pylori* detection in asymptomatic patients with atrophic body gastritis at the primary care level.

Setting: General practice in a rural village in the South-West of The Netherlands.

Methods: The study involved 20 primary care patients with histologically confirmed moderate to advanced atrophic body gastritis which were found by serological screening on hypopepsinogenaemia A and hypergastrinaemia. ^{13}C-UBT and serology were compared as *H. pylori* diagnostics. Culture of a corpus biopsy was considered as reference test for the detection of current *H. pylori* infection.

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R***esults:*** Eight (40%) patients were positive by ^{13}C-UBT and 12 (60%) by serology. Culture of a corpus biopsy established current *H. pylori* infection in 7 (35%). All tests used in the diagnosis of *H. pylori* infection were in agreement in 12 (60%) patients, being all positive in 6 and all negative in 6. One patient (5%) was positive for serology and culture but negative for ^{13}C-UBT, 5 (25%) patients had only positive serology and 2 (10%) patients had only positive ^{13}C-UBT. ^{13}C-UBT showed an accuracy with culture of 85.0% and anti-*H. pylori* serology with culture an accuracy of 75.0 %. The ^{13}C-UBT carried out in the patients with positive serology showed an accuracy of 92%. ROC curve analysis of ^{13}C-UBT demonstrated optimal discrimination at the prescribed cut-off value.

Conclusions: ^{13}C-UBT can be used as diagnostic *H. pylori* test in asymptomatic patients with atrophic body gastritis, preferably in addition to serology, to select subjects for anti-*H. pylori* therapy.

Introduction

To select subjects for *H.pylori* eradication therapy, a definite diagnosis of ongoing *H. pylori* infection should be made. Several methods may be used for the diagnosis of *H. pylori* infection, including endoscopy with biopsy, serological testing, urea breath test and stool assay [1-3]. In general, biopsy-based tests, such as histology, culture and rapid urease test, are recommended when endoscopy is performed. According to the guidelines of the European *H. pylori* study group, ^{13}carbondioxide urea breath test (^{13}C-UBT) or stool antigen test is strongly recommended for the detection of *H. pylori* infection in the initial diagnosis because of sensitivity and specificity scores of 93% [3,4].

Among the indications for *H. pylori* diagnosis and eradication, the European *H. pylori* study group emphasizes atrophic gastritis. Atrophic changes in the gastric mucosa are associated with an increased risk for possible progression to gastric cancer [5-8] and therefore this condition requires intervention by the eradication of *H. pylori* [9,10], although there is no proof that the risk of progression to neoplasia is reduced.

Early diagnosis of atrophic gastritis can be achieved by the primary care physician. By means of a so-called serological gastric biopsy with low serum pepsinogen A, low pepsinogen A/C ratio and with elevated serum gastrin, it is possible to identify persons with atrophic gastritis of the corpus [11,12]. Further aetiological typing of the gastric atrophy by measuring antibodies to *H. pylori* and to parietal cells, is a next diagnostic step and ultimately, biopsy specimens for a histological approach are needed.

There is no "gold standard" test for the detection of *H. pylori*. The accuracy of most diagnostic methods is simply good in subjects with non-atrophic gastritis. The sensitivity and specificity of histological tests are generally more than 90% [13]. Culture and rapid urease tests seem to be less sensitive than histology [14]. Biopsy-based tests, however, explore only a small part of the gastric mucosa, raising the question

of sampling errors. By using the non-invasive tests, ^{13}C-UBT and serology, it is possible to avoid the risks of sampling problems by assessing the entire gastric mucosa. ^{13}C-UBT provides an accurate diagnosis of active *H. pylori* infection [15,16]. It is a so-called active test because it detects current infection [16,17].

Serology is a so-called passive test as it marks exposure to *H. pylori* but does not indicate if active infection is ongoing [16]. The sensitivity and specificity of ^{13}C-UBT and serology exceed generally 90% [4,13].

However, in atrophic gastritis all tests for the diagnosis of *H. pylori* infection, invasive and non-invasive, have their restrictions [18,19]. Detection of *H. pylori* infection in atrophic body gastritis is difficult, as during progression of atrophy, *H. pylori* may be hardly demonstrable, possibly because the non-acidic gastric milieu is unfavourable. Especially in case of extensive intestinal metaplasia *H. pylori* may disappear completely [9]. Therefore, accuracy of invasive diagnostic tests based on gastric biopsies might be restricted if *H. pylori* infection is patchy or if the number of bacteria is low. It is noteworthy that the sensitivity of *H. pylori* histology in atrophic gastritis is without any doubt dependent on the expertise of the pathologist [20].

Also the non-invasive ^{13}C-UBT and serology have an altered feasibility and effectiveness in patients with atrophic gastritis.

UBTs can become false-positive and false-negative in the case of progressive hypochlorhydria due to atrophy or use of acid lowering medication. False-positive results may be due to contamination with non-*H. pylori* urease producing bacteria because the UBT measures urease activity and not the presence of a *H. pylori* infection [21].

False-negative results may be due to possible clearance of the infection in the course of progressive atrophic gastritis, resulting in a lower load of bacteria. Low UBT-values might be associated with more extensive atrophy [18] or even increased risk of gastric cancer [22].

Regarding serology, long-term follow-up studies in patients with advanced atrophic corpus gastritis, showed a spontaneous disappearance of *H. pylori*, as reflected by decreasing and extinguishing antibody titers [23,24]. Patients who initially had elevated serum *H. pylori* antibody levels became seronegative during the 10-year follow-up [24].

Although the widely used ^{13}C-UBT has been repeatedly described, it is remarkable that very few studies have been conducted, so far, to evaluate its diagnostic place in subjects with atrophic gastritis [18,19]. In reviews and editorials upon ^{13}C-UBTs, either nothing is said about ^{13}C-UBT-performance in gastric atrophy or it is dissuaded to use the UBT in patients with atrophic gastritis but without references [21,25]. Therefore, we investigated the effectiveness of ^{13}C-UBT for assessment of *H. pylori* diagnosis in asymptomatic subjects with atrophic body gastritis selected from the general population and used as reference test culture of biopsy specimens obtained by endoscopy. Furthermore we aimed to compare ^{13}C-UBT with serological findings.

Subjects and methods

Study population

The study population comprised 20 patients (11 male, 9 female; age range 31-93 years, mean 68 years; indigenous Dutch citizens, born in The Netherlands) recruited in the family practice in 's-Gravenpolder, a rural village in the Dutch province Zeeland. Nobody of this group had used acid suppressant or antibiotic medication during and before the study; nobody had a documented successful *H. pylori* eradication therapy. They were selected from 997 adult subjects, consecutively entering the primary health care system because of common medical problems, who volunteered in a serological screening study for atrophic body gastritis. Our validated criteria for serological atrophic corpus gastritis were a serum concentration of pepsinogen A < 17µg/l, a pepsinogen A:C ratio < 1.6 and an accompanying serum concentration of gastrin > 100 ng/l [26,27]. Serological atrophic corpus gastritis was established in 34 patients. A total of 25 of those 34 patients agreed in undergoing upper gastrointestinal endoscopy with biopsy. Ultimately 20 persons appeared to have histological gastric corpus atrophy, based on the full spectrum of the updated Sydney classification system.

The local Ethics Committee approved the study. All participating subjects gave informed consent before entering the study.

Detection of *H. pylori*

Non-invasive tests for detecting *H. pylori* infection

- ***^{13}Carbon urea breath test***

The ^{13}C-UBTs were performed at the general practice office by the main investigator himself (AK). All 20 participants in the study population were tested with the EMEA approved INFAI ^{13}C-urea breath test, a German test from the Institute for biomedical Analysis in Bochum. The test was performed according to the manufacturer instructions. Operator familiarity with ^{13}C-UBT was present with minimum opportunity for methodological error.

Patients were invited to come to the GP-office after an overnight fast. The test was started with blowing through a straw into a 10 ml glass tube with a stopper. This provided the baseline sample. Also a second baseline sample container with breath in the same way was filled up. Next, they were asked to drink a sachet of orange juice of 200 ml to delay gastric emptying. Next, they consumed a drink containing 75 mg ^{13}C enriched urea (30 ml) and after 30 minutes repeated the blowing exercise into the last 2 test-containers. This provided the post-dose samples. All the 4 breath samples were sent away for $^{13}CO_2/^{12}CO_2$ ratio analyses by mass spectrometry of the isotopometric ratios rs of $^{13}CO_2/^{12}CO_2$ versus that of a reference gas (r_r).

The δ-value is expressed as: $\delta[‰] = ((r_s - r_r) / r_r) \times 1000$. The change on δ-value 30 minutes after exposure to ^{13}C –urea is then $\Delta\delta = \delta_t - \delta_0$ and is considered positive if the mean value exceeds 4.0 ‰. The larger Δδ, the larger the extent of the infection.

- Serological H. pylori test

All serum samples were serologically tested for *H. pylori* in the research laboratory of the department of Gastroenterology and Hepatology of the Leiden University Medical Centre by a validated enzyme immunoassay detecting specific immunoglobulin G against a homogenate of 6 strains of *H. pylori*. Western blots of this homogenate showed the presence of CagA bands, indicating a cytotoxic variety of *H. pylori*. The results were expressed as the absorbance index (AI) of the sample versus a reference serum: serum with an AI>0.32 IgG *H. pylori* antibody was considered positive [28].

Invasive *H. pylori* tests

- Endoscopy and biopsy

Gastroscopy had been performed in the usual manner using Olympus video-endoscopy equipment. Antral and fundic biopsy specimens were systematically collected as follows: 6 biopsies from the mid antrum, about 2 cm pre-pyloric from the anterior and posterior antral wall, 4 for histological examination, 2 for culture; 6 biopsies from the mid body, about 5 cm distal of the oesophagus-cardia junction from the anterior and posterior body wall, also 4 for histology and 2 for culture. Biopsies for histology were fixed in 10% formalin, biopsies for culture in physiologic saline solution.

- Culture of H. pylori

Biopsies were transported in saline and smeared on selective agar (Columbia agar, supplemented with 7% lysed horse blood and a selective antibiotic mix with Vancomycin, Cefsulodin, Trimethoprim and Amphotericin B all from Oxoid B.V., Haarlem) and incubated for a maximum of 5 days at 37° C in a micro-aerophilic environment. Typical colonies, detected at daily inspection, were examined with Gram staining, and if they had a typical spiral morphology, were confirmed using urease, oxidase, and catalase detection.

- Histology of H. pylori

In brief, 5 micron sections were hematoxylin and eosin stained and examined according to the updated Sydney classification system. Additional Giemsa staining and immunostaining with antibodies against *H. pylori* were performed for optimal identification of the micro-organism.

Statistical analyses

Statistical analyses were performed with Pearson chi-square and the Fisher's exact test. P values <0.05 were considered significant. The receiver operating characteristics (ROC) curve and statistics were calculated by SPSS® 12.0.1 for Windows®.

Table 1. *Characteristics of 20 primary care patients with atrophic body gastritis*

Pat	Gender and age (year)	^{13}C-UBT $\Delta\delta$ >4.0 ‰ = +	Anti-Hp antibodies AI >0.32 =+	Hp-*culture* antrum / corpus	Hp-*histology* antrum / corpus	Grade of corpus *atrophy*	Inflamm corpus *active/ chronic*	Grade of corpus *intestinal metaplasia*	Grade of antrum *atrophy*
1	F – 53	- ve 0.33	- ve 0.15	– / –	– / –	+++	+/++	+	–
2	F – 31	- ve 1.53	- ve 0.08	– / –	– / –	+++	+/++	+	–
3	M- 75	+ ve7.90	+ve 0.93	– / +	– / –	+++	+/++	+++	–
4	F – 66	- ve 1.15	- ve 0.03	– / –	– / –	+++	–/++	++	–
5	M- 74	+ve11.62	+ve 0.69	+ / +	+ / +	++	++/++	+	–
6	M- 57	- ve 2.26	+ve 0.48	– / –	– / –	+++	–/++	++	–
7	M- 79	- ve 3.44	- ve 0.24	– / –	– / –	+++	+/++	++	–
8	F – 45	+ve 11.7	- ve 0.20	– / –	– / –	+++	+/+++	+	–
9	M- 78	+ve 12.5	- ve 0.08	– / –	– / –	+++	+/++	+++	–
10	F – 80	- ve 1.86	+ve 0.66	– / –	– / –	++	+/++	+	–
11	M- 72	+ve 31.0	+ve 1.04	– / +	+ / +	+++	+/+++	+++	+++
12	M- 83	- ve 0.00	+ve 0.60	– / –	– / –	++	+/++	+	–
13	F – 85	- ve 1.13	- ve 0.31	– / –	– / –	++	–/++	+	–
14	M- 60	+ve 9.47	+ve 0.83	+ / +	+ / +	++	++/+++	+	+
15	F – 70	- ve 2.28	+ve 0.41	– / –	– / –	++	–/++	+	–
16	F – 39	- ve 1.78	+ve 0.35	– / –	– / –	+++	+/++	++	–
17	F – 74	+ve 10.7	+ve 1.12	– / +	+ / +	++	++/+++	++	+
18	M- 70	- ve 3.26	+ve 0.79	– / +	+ / +	++	+++/+++	++	++
19	M- 93	- ve 3.26	- ve 0.18	– / –	– / –	+++	–/++	+	++
20	M- 73	+ve 17.1	+ve 0.45	– / +	– / +	+++	+/+++	+++	+++

^{13}C-UBT = ^{13}carbon urea breath test
anti-Hp = antibodies to *Helicobacter pylori*
Hp-culture = culture of biopsy specimens for *Helicobacter pylori* infection
Hp-histology = histological investigation of gastric mucosa specimens for *H. pylori*
– = absent; + = mild ; ++ = moderate; +++ = severe

Results

Twenty patients with serological and histological confirmed atrophic body gastritis, could be analysed *(see Table I)*.

Grade of atrophy and intestinal metaplasia *(see Table I)*
The grade of atrophy of the corpus mucosa at the time of the study was moderate in 8 and severe in 12 subjects. None of the subjects scored mild atrophy. Corresponding atrophy scores for the antrum were, respectively mild 2 persons, moderate 2 and se-

Figure 1. *Comparison of the diagnostic value of ¹³C-UBT(‰) and serology of anti-H. pylori antibodies. The horizontal line shows the cut-off value (Δδ <4.0 ‰) of ¹³C-UBT and the vertical line the cut-off value of anti-H. pylori (Absorbance Index <0.32). Closed points are patients with a culture of corpus biopsies positive for H. pylori infection. Open points are cultured negative.*

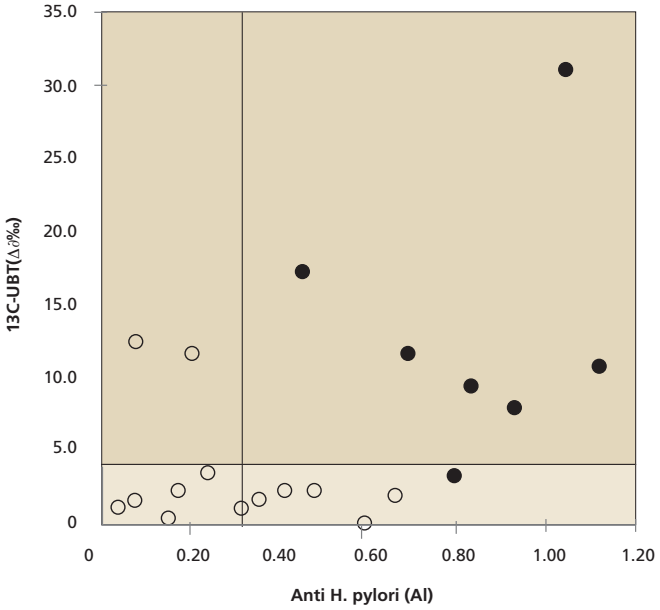
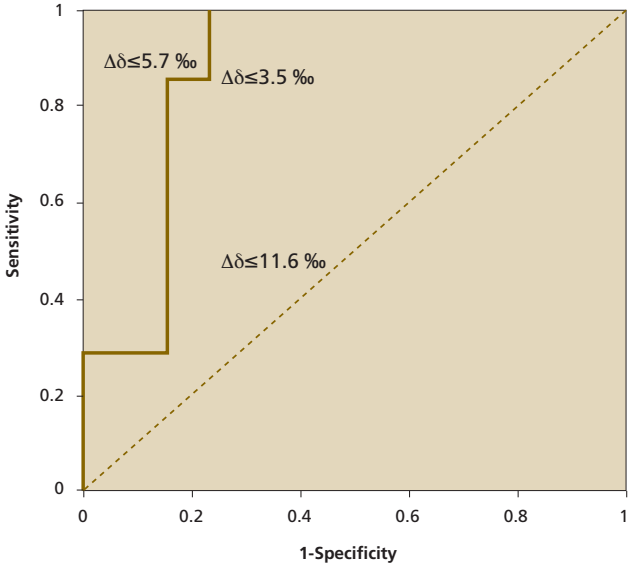


Figure 2. *ROC curve of detection of H. pylori infection by ¹³C-UBT. At the angle points Δδ limits have been signed.*



vere also 2 persons. Intestinal metaplasia (IM) of the corpus mucosa was severe in 4 and moderate in 6 patients, whereas mild or no IM was seen in 10 patients.

***H. pylori* status** *(see Table I)*

^{13}C-Urea breath test

^{13}C-UBT was positive in 8 patients (40%) of 20.

Culture and histology

H. pylori culture was positive in 7 (35%) patients and in 6 of them histology was positive.

Serology

12 (60%) of 20 patients showed serum antibodies to *H. pylori.*

All 7 patients with positive culture had antibodies to *H. pylori* with Absorbance Indexes ranging from 0.45 to 1.12. The remaining 5 patients showed AI's from 0.35 to 0.66.

***H. pylori* prevalence in different grades of atrophic corpus gastritis**

H. pylori culture was positive in 4 of 7 (57%) patients with *moderate* corpus atrophy and in 3 of 7 (43%, P =0.36, Fisher's exact test) patients with severe corpus gastritis, respectively.

H. pylori serology was positive in 7 (88%) of 8 patients with *moderate corpus* atrophic gastritis and in 5 of 12 patients (42%, P =0.07, Fisher's exact test) with severe atrophic gastritis.

^{13}C-UBT suggested infection in 3 (37.5%) of 8 patients with *moderate* atrophy and in 5 of 12 (42%, P =1.00, Fisher's exact test) with severe atrophic corpus gastritis. Infected patients had higher scores of active and chronic corpus gastritis ($p < 0.05$).

Diagnosis of *H. pylori* infection based on combination of tests *(Figure 1)*

All tests used in the diagnosis of *H. pylori* infection were in agreement in 12 (60%) patients, being all positive in 6 (30%) and all negative also in 6 patients. Five (20%) had only positive serology. Two(10%) had only positive ^{13}C-UBT.

Accuracy

^{13}C-UBT had an accuracy with culture of 85% (17 of 20), while the accuracy of anti-*H. pylori* serology with culture was 75% (15 of 20). However, when the ^{13}C-UBT was carried out only in the 12 patients with positive serology, the accuracy of ^{13}C-UBT was 92%.

ROC curve *(Figure 2)*

The standardised area under the curve is 0.88 (S.E.M = 0.08). The asymptotic P is <0.01 so that the area differs significantly from the null hypothesis: true area = 0.5.

Discussion

The ^{13}C urea breath test is routinely used for diagnosing and confirming eradication of *H. pylori* after therapy. The appropriateness of ^{13}C-UBT in subjects with atrophic gastritis is hither'to scarcely described [18,19] and fairly unknown.

The present study therefore aimed to assess the performance of the ^{13}C-UBT in subjects with histologically confirmed atrophic corpus gastritis. Our UBT-study was evaluated totally in an average primary care population and all UBT-tests were performed by the general practitioner himself. Our study population totally differs from earlier studies [18,19] on the value of ^{13}C-UBT in atrophic gastritis. The study of Kokkola et al. [18] was part of the Alpha-Tocopherol, Beta Carotene Cancer Prevention Study and selectively included elderly male smokers. In another study [19] patients with anaemia and/or long-standing dyspepsia were enrolled.

In general, it can be said that ^{13}C-UBTs are very accurate tests for detecting H pylori with a sensitivity and specificity better than many other tests [4,16,21].

However, in the package leaflet with patient documentation of the INFAI ^{13}C-UBT, one can read that the test must not be used in patients with documented or suspected atrophic gastritis, which might interfere with the urea breath test. In the leaflet there is no concrete reference to underpin this warning.

Keeping in mind that atrophic gastritis, a risk factor for gastric cancer, is a late consequence of *H. pylori* infection in approximately one-third of the infected patients, and assuming that gastric cancer would develop less frequently if *H. pylori* were eradicated, it is important to detect and cure *H. pylori* infection in atrophic conditions of the stomach [3-5]. Nevertheless, all diagnostic *H. pylori* tests, invasive and not-invasive, may have failure results in atrophic gastritis. To date, in atrophic gastritis, biopsy-based *H. pylori* diagnostic methods are very prone to sampling problems, serology may be positive in both ongoing and past *H. pylori* infection, UBTs can give false-positive and negative results, respectively due to contamination with non-*H. pylori* urease producers and insufficient bacterial load. So, a combination of tests is warranted in atrophic gastritis to avoid underestimation of *H. pylori* infection prevalence.

Our study shows that the 3 cornerstones of *H. pylori* diagnostic, i.e. UBT, serology and culture, did perform better than could be expected in patients with atrophic gastritis.

Overall, it appeared to be possible to detect previous and current *H. pylori* infection in 60% (12 versus 20) whereas in the remaining 40% of the group an autoimmune aetiopathogenesis of the atrophy should be assumed.

In the field of *H. pylori* diagnostics in atrophic gastritis, ^{13}C-UBT as indicator of current infection can play an important role; it approaches the sensitivity of culture (86%) and could certainly serve as an additional test reflecting the entire mucosa, being not prone to sampling errors. When the ^{13}C-UBT was carried out only in the 12 patients with positive serology, the accuracy appeared to be 92%. Our study in-

dicates that the combination of serology and ^{13}C-UBT, could be used in diagnosing or excluding ongoing *H. pylori* infection in atrophic gastritis. These test results could be starting point for therapeutic decisions.

In this context, a relevant paper by Kokkola and colleagues in this issue reports a study in which they describe that serology is more reliable to estimate active *H. pylori* infection in symptomatic patients with atrophic gastritis than ^{13}C-UBT and histology [29]. They found that patients with atrophic corpus gastritis and elevated *H. pylori* antibody titers but ^{13}C-UBT and histology-negative for *H. pylori*, after randomizing into eradication therapy or follow-up only, showed significantly decreasing titers in the eradication group compared with the follow-up subjects. So, positive serology results may indicate ongoing infection in spite of negative UBT and histology [29]. However, the selected secondary care population in the study of Kokkola is quite different compared to our primary care subjects as sample of the general population.

Performance of ^{13}C-UBT

The INFAI ^{13}C-UBT appeared to be a feasible test for use in primary care and operator familiarity is rapidly acquired. As mentioned above, in our study in patients with atrophic gastritis, the concordance of ^{13}C-UBT with culture as reference, appeared to be 86% (6 versus 7). Comparison of ^{13}C-UBT with serology showed a concordance of 50% (6 versus 12). Based on these study results, it is clear that the ^{13}C-UBT performs fairly well but is not suitable as a single decisive test in atrophic conditions of the gastric mucosa. It should at least be combined with serology. Noteworthy are patients nos. 8 and 9 with a clearly positive ^{13}C-UBT-score but with negative serology and culture. Both patients have severe corpus atrophy and respectively mild and severe intestinal metaplasia. One patient is only 45 years old, so relatively young to have already extinguished *H. pylori* serology. Moreover, both patients have autoimmune positive serology. It is possible that the ^{13}C-UBT-results may be false positive, attributable to non-*H. pylori* urease producers. On the contrary, patient no. 18 might have a false negative ^{13}C-UBT because of positivity of both culture/histology and serology. He and his general practitioner and pharmacist (AK) denied the use of acid-suppressive medication, sucralfate or antibiotics in the last 10 days before undergoing the test, as common causes of a false negative ^{13}C-UBT. Low gastric acid due to antisecretory drugs is reported to lead to false negative ^{13}C-UBT results [30-33]. Extrapolating these data to patients with gastric atrophy who have no or negligible acid secretion suggests that there is no place for ^{13}C-UBT test in patients with gastric atrophy.

Consequences for a diagnostic strategy of *H. pylori*-related atrophic gastritis.

The appropriateness of ^{13}C-UBT and endoscopy referrals by primary care physicians leaves much to be desired and the management of *H. pylori* infection still requires educational programmes [34,35].

The Maastricht 2-2000 consensus report recommends that *H. pylori* in patients with *H. pylori*-related atrophic gastritis should be eradicated to prevent ongoing atrophic detriment. Detection of gastric atrophy and *H. pylori* can be achieved by the general practitioner and should be done in subjects at risk, i.e. first degree relatives of patients with gastric cancer [36]. The diagnostic tools are the serological gastric biopsy, *H. pylori* serology and ^{13}C-UBT. With optimal use in primary care setting of non-invasive diagnostic methods it is possible to make a good selection of subjects to refer for endoscopy and to target cost-effectiveness in health care.

In conclusion, for the selection of patients for *H. pylori* eradication therapy in primary health care setting we encourage to use ^{13}C-UBT test as *H. pylori*-diagnostic test in patients with atrophic gastritis and we advise to combine ^{13}C-UBT with serology for optimal detection of current and previous infection. We recommend in atrophic gastritis the use of ^{13}C-UBT only for patients with positive serology. This approach to select patients with atrophic gastritis for *H. pylori* eradication therapy combines high efficiency with high accuracy. The approach to use the ^{13}C-UBT only in patients with positive *H. pylori* serology probably also applies to patients with acid-peptic disease using antisecretory agents, such as proton pump inhibitors or H_2-receptor antagonists.

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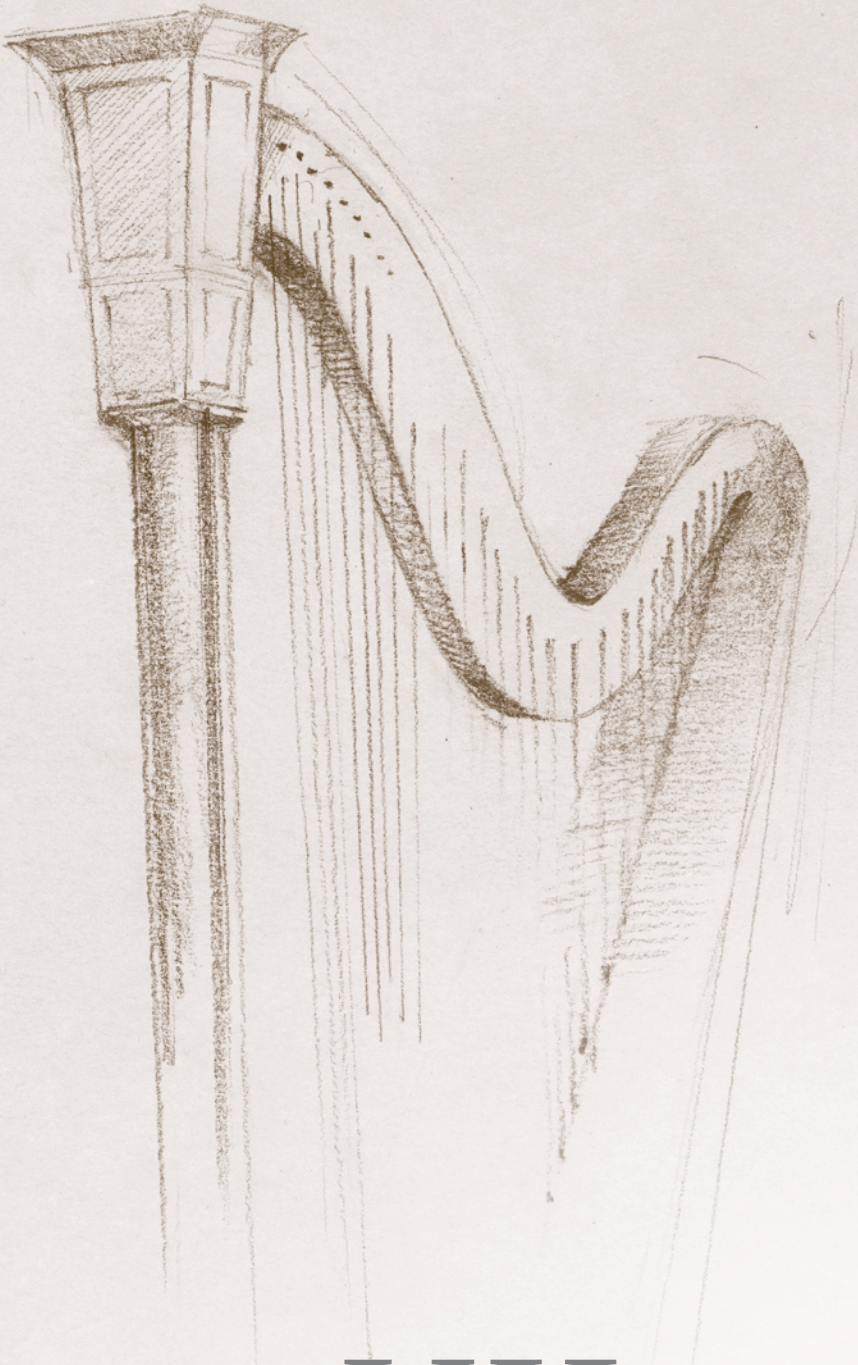

Chapter VII

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Beauty is in the eye of the beholder.

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Chapter VII

Short-term proton pump inhibitor administration in the non-invasive diagnosis of the grade of atrophic corpus gastritis

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Keywords: Atrophic corpus gastritis, Proton pump inhibitor stimulation test, Pepsinogen, Gastrin

Abstract

Background: *Proton pump inhibitor (PPI) administration is known to be a potent pepsinogen-releasing stimulus in healthy volunteers. Pepsinogen A level decreases in patients with atrophic changes in the gastric corpus. A decreased serum pepsinogen A respons to PPI, as a marker of an impaired oxyntic chief cell function, may be used as a refined atrophy test of the oxyntic mucosa.*

Aim: *To verify the hypothesis that in patients with atrophic corpus gastritis PPI-induced increase of pepsinogen A is inversely correlated with the grade of atrophy.*

Patients: *Study subjects were 25 primary care patients with serological atrophic corpus gastritis (12 M, 13 F, mean age 67 years, range 31-93), outcome of a community based explorative study.*

Methods: *After obtaining pre-treatment fasting sera and biopsy specimens via upper endoscopy, esomeprazole was administered to all 25 patients during 2 weeks, 40 mg daily. The grade of atrophy in the biopsy tissue specimens was assessed according to the updated Sydney system. An after-treatment fasting blood sample was collected for evaluation of serum pepsinogens and gastrin levels. Finally, the serum pepsinogen A-respons to PPI was related to the histology of each patient.*

Results: *At pre-treatment testing, 20 of the 25 patients again fulfilled the criteria of*

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serological atrophic corpus gastritis. Pre-treatment histological examination of corpus biopsies of the whole group revealed 13 patients with severe, 6 with moderate and 1 patient with mild gastric body atrophy. One patient had no corpus biopsies. After 2 weeks PPI-administration the 19 patients with moderate to severe atrophic body gastritis showed negligible increase in pepsinogen A serum level (mean preentry level 4.7 μg/l and post-PPI 4.9 μg/l, $p > 0.05$) and 1 patient with mild atrophy showed slight increase in pepsinogen A concentration (preentry 16, vs post-PPI 26). Post-PPI serum gastrin levels had no additional diagnostic value in grading gastric body atrophy. As expected, 4 patients with normal serum profile showed in the biopsies no gastric corpus atrophy. After PPI-administration they had significant, three- to fourfold, increase of the concentrations of pepsinogen A and a marked increase of serum gastrin from normal baseline ($p < 0.05$).

***Conclusion:** PPI-stimulated increase of pepsinogen A in patients with atrophic corpus gastritis has an inverse relation with the grade of atrophy ($r = - 0.79$, $p < 0.001$). Stimulated pepsinogen A response to PPI can therefore be used as a marker for the severity of atrophic body gastritis.*

Introduction

Since the recognition of gastric atrophy in 1870 – in post-mortem samples from a patient with pernicious anaemia – much attention and research have been devoted to decipher its underlying pathophysiology [1]. The biological background of the development of gastric atrophy is still incompletely understood. Chronic atrophic gastritis is a multifactorial condition, caused by the interplay between a genetic set-up, gastric bacterial infection, and environmental factors [2-6]. Atrophic gastritis is considered to be a preneoplastic lesion [7-9] and therefore reliably diagnosing and grading of gastritis and mucosal atrophy is an important health issue.

Gastric endoscopy with biopsy is the typical diagnostic method to determine atrophic gastritis, but this is not done routinely in patients without gastric cancer. Its principal use is to rule out the presence of cancer rather than to determine the presence and extent of atrophic gastritis.

Serum pepsinogen A and C have become popular as indicators of atrophic body gastritis in epidemiological studies because these determinations are more simple and less invasive than endoscopy with biopsy. A comparison between the histological findings of atrophic gastritis and the serum pepsinogen levels has already been made in the 80-s of the last century [10].

Serum pepsinogen assays identify the majority of patients with atrophic corpus gastritis, although they are less useful in assessing the degree of atrophy in detail. It is reported that the agreement of the extent of atrophic gastritis, assessing by endoscopic examination and by serum pepsinogen levels, was 57%, and agreement of the presence of atrophic gastritis was 79% [11]. Depending on the population,

pepsinogens often have a high specificity but low sensitivity for the diagnosis of atrophic gastritis, whereas antibodies against *Helicobacter pylori* or CagA have a high sensitivity but low specificity [12,13]. Serological techniques, in fact, suffer from an intrinsic limitation related to their low sensitivity for the mild forms of atrophic gastritis [11]. This can be deduced from the fact that most of the commonly used assays are based on the determination of more than one parameter to compensate for this limitation, and often it is clearly indicated that their use is confined to the assessment of "moderate-severe" or "advanced" forms of atrophy [14-16].

The histological diagnosis remains the gold standard in the assessment of gastric atrophic pathology. However, even the histological diagnosis of chronic atrophic gastritis is sometimes questionable, particularly in the context of active inflammation [17,18]. Also the patchy distribution of atrophic disorders may be responsible for false-negative results because biopsies might be taken from areas not involved by the disease. This may be of great consequence because atrophic gastritis is an important risk factor for gastric cancer [19,20].

To tackle the problem of the poor diagnostic performance of serological and histological methods for the diagnosis of fundic atrophic gastritis, complementary tests are mandatory. Recently a novel device for gastric juice analysis, measuring ammonium concentration and pH during endoscopy, seems to be a promising tool for overcoming this diagnostic deficiency [21].

However, considering the physiological concept that secretory behaviour reflects trophic condition and that secretory stimulation is a well-known diagnostic test to investigate integrity of functional cell mass, it is reasonable to assume that reduced secretion of pepsinogen after stimulation might be a refined diagnostic marker of atrophy of the gastric corpus. Proton pump inhibitor (PPI) administration is known to be a potent pepsinogen-releasing stimulus in healthy volunteers [22-24]. Our premise is that in patients with atrophic corpus gastritis with loss of chief cell-mass, PPI-stimulation has a more or less rising effect on the level of serum pepsinogen, depending on the severity and extent of cell-reduction. In case of mild atrophy one could expect a modest increase in serum pepsinogen level after stimulation; in case of advanced atrophy there will be no relevant pepsinogen increase.

Therefore, a non-invasive PPI-stimulation test might give clearness in the trophical darkness of the gastric mucosa and may circumvent the problem of invasiveness of endoscopy and gastric juice analysis.

The present study was undertaken to test the hypothesis that patients with corpus mucosal atrophy show abnormally low pepsinogen responses to PPI-administration. Therefore, we have measured serum pepsinogen levels in patients with serological body atrophy before and after 2 weeks course of esomeprazol 40 mg daily. In addition we have compared this individual pepsinogen response to PPI with the histological data of the biopsy specimens of each patient. To the best of our knowledge, this is the first report on the diagnostic performance of short course PPI to determine the severity of atrophic body gastritis and possibly the increased

risk for gastric cancer. Our study premise is that PPI-induced increase of pepsinogen A in patients with gastric body atrophy has an inversely graded relation with atrophy.

Subjects and methods

Study Population

The study was performed in a group of 25 patients with serological gastric body atrophy. They were selected from 997 adult subjects, consecutively entering the primary health care system because of common medical problems, who volunteered in a serological screening study for atrophic body gastritis. Subjects younger than 18 years, pregnant women, patients with gastric resection, renal insufficiency and those using antisecretory agents were excluded. All 25 study subjects, 12 men and 13 women, mean age 67 years, range 31-93, were indigenous Dutch citizens, born in The Netherlands. Nobody of this group of 25 had used acid suppressant medication before the study.

The study was performed in the family practice in 's-Gravenpolder, a rural village in the province Zeeland in The Netherlands.

Informed consent was obtained from all subjects, and the protocol was approved by the Ethics Committee of the Leiden University Medical Center.

PPI-choice, dosage regimen and testing scheme

Esomeprazole 40 mg, was administered orally, once daily for 14 days. The choice for esomeprazole, the modern S-isomer of omeprazole, was made by the literature based outstanding sustained gastric acid control with reduced interpatient variability [25-27]. A dosis of 40 mg daily appears to be the dosis for optimal and safe acid suppression. Before and after the 2 weeks esomeprazole course venous blood samples were collected from all subjects for the assessment of fasting serum pepsinogen and gastrin.

Serological examination

All sera samples were tested by well-validated radio-immunoassays for levels of pepsinogen A, pepsinogen C and gastrin [28]. Our validated criteria for advanced serological atrophic corpus gastritis, corresponding to pentagastrine refractory achlorhydria or severe hypochlorhydria (PAO < 5 mmol/hr), expressed in the level of the serum markers, were a serum concentration of pepsinogen A < 17 μg/l, a pepsinogen A/C ratio < 1.6 and an accompanying serum concentration of gastrin > 100 ng/l [28].

Endoscopy and biopsy sampling

Before the start of the PPI-administration all individuals underwent endoscopy in

the usual manner, using an Olympus video-endoscopy equipment. Standardized biopsy sampling for histological examination was performed with 4 biopsies from the anterior and posterior wall of the mid antrum and also 4 biopsies from the anterior and posterior wall of the mid corpus, respectively about 2 cm pre-pyloric and about 5 cm distal of the oesophagus-cardia junction. Biopsies for histology were fixed in 10% buffered formalin.

Histological examination

In brief, 5 micron sections were hematoxylin and eosin stained and examined according to the updated Sydney classification system [29-31]. Atrophy was defined as loss of the deeper specialized glands, i.e. in the gastric body the parietal cells and secondly the chief cells, in the antrum the gastrin producing G-cells. Intestinal metaplasia (IM) is the replacement of gastric mucosa by small intestine-like mucosa. IM contributes to atrophy when extending deeply into the mucosa to involve the gland compartment. All histopathological issues were semi-quantitatively graded as absent, mild, moderate or severe.

Statistical analysis

Statistical analysis of the differences before and after esomeprazol administration was performed by applying the paired Student's t-test and Pearson product moment correlation coefficient.

Results

Retesting Serological atrophic corpus gastritis before PPI-Administration

Based upon the pretreatment testing of the biomarkers, 20 patients, of the group of 25, again fulfilled the criteria of serological atrophic corpus gastritis, while 5 participants did not.

The 5 "drop-outs" subjects showed the following serological characteristics. One subject (no. 8) had after retesting normal gastrin with low pepsinogen A and low ratio A:C, thus no longer fulfilling the criteria of serological gastric body atrophy. The remaining 4 subjects showed after retesting normal serum profile, of whom 2 persons (nos. 3 and 14) had borderline test results in the first round and the other 2 persons (nos. 6 and 15) had an unexplained conversion to normal levels of the serum markers. Retesting is mandatory in case of borderline test results.

Pretreatment histological examination of oxyntic and antral Mucosa

The presence or absence of atrophic gastritis and the grade of atrophy was assessed directly before starting PPI-administration.

As shown in *table 1*, histological atrophic body gastritis was found in 20 patients, of whom 1 subject scored mild, 6 persons moderate and 13 severe body at-

rophy. One patient (no. 4) with serological atrophic corpus gastritis had only antrum biopsies and no corpus biopsy specimens. Nine patients showed histological antral atrophy, 5 mild, 2 moderate and 2 severe atrophy. One patient had no antral biopsies (no.15).

Four patients (nos. 3, 6, 14 and 15) showed only chronic aspecific inflammation and no atrophy.

Serum pepsinogen A concentration before and after PPI-stimulation

The individual measurements of pepsinogen A before and after the 14-day course of oral esomeprazole are shown in *table 1* and *figure 1*.

In the 4 non-atrophic patients (patient nos. 3, 6, 14, 15), mean age 65 years (range 49-77), a marked increase in the pepsinogen A level occurred: mean pepsinogen pre-entry level 17 μg/l, mean post-PPI concentration 59 μg/l, $p < 0.05$.

The 19 patients with moderate and severe histological atrophic corpus gastritis, mean age 58 years (range 31-93) showed negligible increase in serum pepsinogen A level. The mean preentry level of pepsinogen A was 4.7 μg/l and the mean post-PPI concentration was 4.9 μg/l, $p > 0.05$ vs. preentry. There was no difference in stimulated pepsinogen levels between patients with moderate and advanced atrophy.

Also patient no. 4, without corpus biopsy specimens, had a negligible change in pepsinogen A level, pointing to severe corpus atrophy.

One patient (patient no. 8), age 74 years, with a borderline pepsinogen A level of 16 μg/l, showed a modest pepsinogen increase, from 16 to 26 μg/l. He appeared to have mild corpus atrophy.

Serum gastrin concentration before and after PPI-stimulation

Serum gastrin levels measured before and after esomeprazole administration are shown in *table 1* and *figure 2*.

Basal serum gastrin concentrations ranged from 59 to 134 ng/l in patients with normal antral and corpus mucosa (patients nos. 3,6,14). Oral PPI-stimulation increased their gastrin very clearly with a range from 602 to 1531 ng/l , $p < 0.05$. One patient (no. 15) had no antrum biopsies but only corpus biopsies; the corpus biopsies showed no atrophy. The gastrin behaviour (preentry 77, post-PPI 760) points to normal antral function.

The group with antral atrophy showed a preentry gastrin range from 68 to 1284 ng/l. The post-PPI range was 122 to 731 ng/l. The presence or absence of corpus atrophy determines in the nature of things also the preentry level of gastrin.

A high preentry gastrin level, pointing to functional intact antral mucosa and an advanced corpus atrophy, remained high after PPI-stimulation with unpredictable increase or decrease.

Patient no. 8 had antral atrophy with metaplasia (preentry gastrin 68, stimulated gastrin 102) and mild corpus atrophy (preentry pepsinogen A 16 and stimulated pepsinogen 102).

Table 1. *Serological and histological characteristics in gastric atrophy in 25 primary healthcare patients before and after 2 weeks PPI-administration*

	pre–PPI serology					post–PPI serology				histology	
patnr	PgA	PgC	A/C ratio	Gastrin	Serol GBA	PgA	PgC	A/C ratio	Gastrin	Histol GBA	Histol GAA
1	1	4	0.3	1664	+	2	4	0.6	1531	+++	–
2	2	9	0.2	1269	+	1	8	0.2	1490	+++	–
3	17	6	2.7	86	–	58	17	3.5	802	–	–
4	1	4	0.3	2212	+	2	6	0.4	2031	■	–
5	3	7	0.4	123	+	3	4	0.8	342	+++	–
6	25	15	1.7	134	–	62	23	2.7	1531	–	–
7	8	9	0.9	933	+	5	8	0.7	1023	+++	–
8	16	23	0.7	68	–	26	22	1.2	102	+	+
9	1	17	0.1	764	+	1	18	0.1	656	+++	–
10	2	12	0.2	534	+	2	9	0.2	642	+++	–
11	0	7	0.1	2086	+	1	6	0.1	2000	+++	–
12	1	2	0.4	1352	+	1	3	0.3	1456	+++	+
13	3	11	0.3	1791	+	3	9	0.3	1385	++	–
14	17	5	3.5	59	–	91	26	3.5	602	–	–
15	9	2	5.3	77	–	24	5	4.3	760	–	◆
16	3	5	0.7	116	+	7	12	0.6	122	+++	+++
17	9	11	0.8	628	+	9	16	0.5	1559	+++	–
18	3	4	0.7	552	+	5	5	0.9	1254	++	+
19	5	16	0.3	459	+	8	11	0.7	403	++	+
20	6	4	1.3	146	+	8	6	1.4	482	++	–
21	1	4	0.3	2257	+	1	5	0.2	1670	+++	–
22	14	18	0.8	1179	+	12	7	1.9	1092	++	+
23	4	12	0.3	159	+	7	9	0.8	261	++	++
24	6	11	0.5	1284	+	7	11	0.6	731	+++	++
25	11	14	0.8	284	+	14	8	1.7	262	+++	+++

■ = no corpus biopsy
◆ = no antrum biopsy

PPI = proton pump inhibitor
PgA = pepsinogen A
PgC = pepsinogen C
A/C rat = pepsinogen A to C ratio
Serol GBA = serological gastric body atrophy
Histol GBA = histological gastric body atrophy
Histol GAA = histological gastric antrum atrophy
– = absent, + = mild, ++ = moderate, +++ = severe

Overall, there were marked differences in the gastrin PPI-response without uniform correlation with the grade of atrophy.

A specific diagnostic pattern of stimulated gastrin is only visible in patients with non-atrophic gastritis of body and antral mucosa.

Correlation between PPI-test and histology

Stimulated pepsinogen A appears to have a reliable predictive value in the grade of atrophic corpus gastritis. Stimulated gastrin shows in the nature of things erratic movements and has no decisive role in the diagnosis of atrophic body gastritis.

One patient had no corpus biopsies, so that in our study in 96% (24/25 patients) PPI-stimulated pepsinogen A and histology matched a similar diagnosis.

PPI-stimulated increase of pepsinogen A in patients with atrophic corpus gastritis has an inverse relation with the grade of atrophy ($r = -0.79$, $p < 0.001$).

An abnormally low pepsinogen A respons to PPI points to severe mucosal atrophy of the oxyntic mucosa.

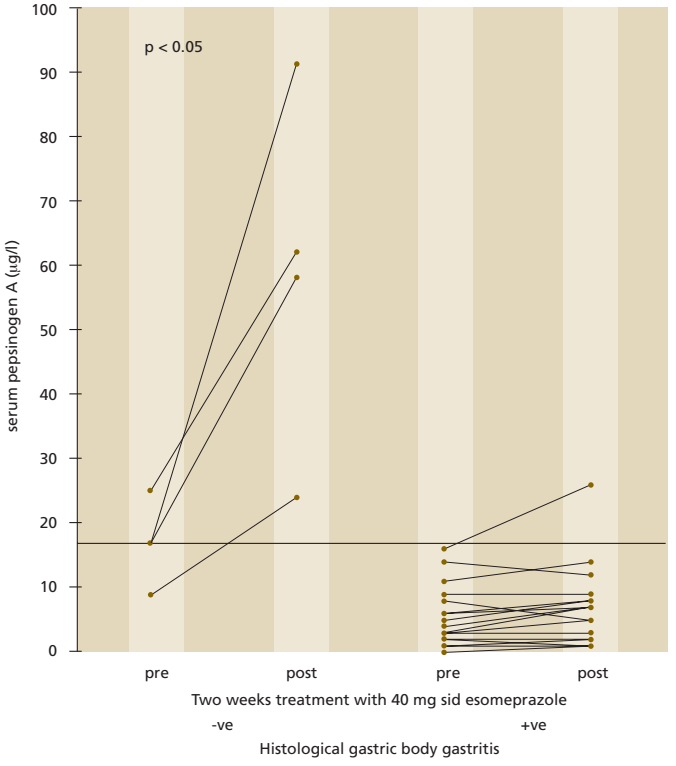

Figure 1.

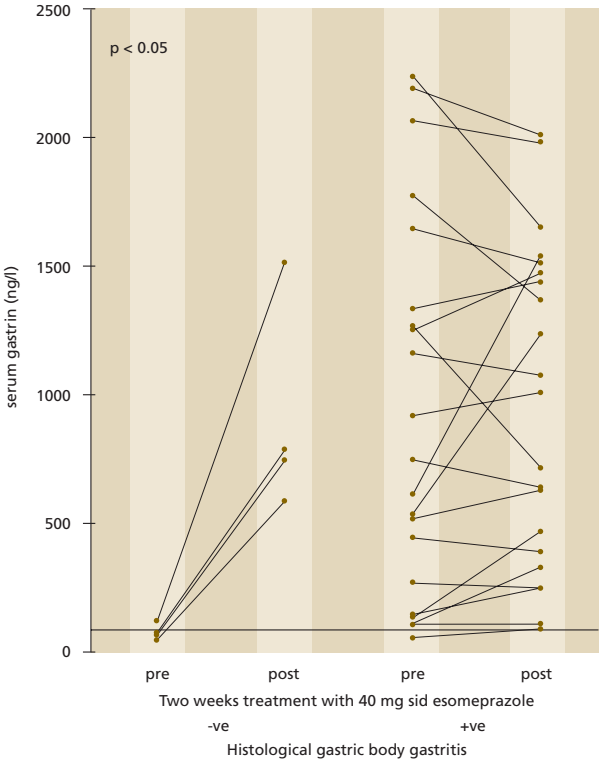

Figure 2.

Discussion

Proton pump inhibitors (PPIs) are highly effective drugs that have revolutionized the management of acid-pepsin disorders over the last two decades. The design of an effective therapy may be useful not only in treatment but also in identifying causes of disease. The selectivity of PPIs is so accurate that this quality can be utilized to identify acid-related disorders, such as gastroesophageal reflux disease [32-35]. Using the "cause-and-effect" respons to a PPI in acid hypersecretion it might be conceivable, from a scientific point of view, to use this respons also in acid hyposecretion of the gastric mucosa. Acid hyposecretion, hypochlorhydria, reflects a degree of atrophy of the stomach and is as a rule asymptomatic. The "cause and effect" response to PPI in gastric atrophic conditions should therefore be considered as a biochemical respons. Preferably non-invasive diagnostic tools are welcome in the often tough diagnosis of atrophic gastritis.

This present work is the first report on the diagnostic approach of atrophic gastritis with PPI- stimulated pepsinogen and gastrin. The main findings in our PPI-atrophy study in patients with atrophic body gastritis are as follows:

[1] increase in PPI-stimulated serum pepsinogen A has an inverse relation with the grade of mucosal body atrophy, [2] gastrin respons to PPI adds no decisive information about the grade of atrophy of the gastric corpus [3] reduced gastrin respons to PPI might be usefull in the case of predominant antral atrophic gastritis (patient no. 8).

The results of the 25 patients, enrolled in this study, indicate that measurement of PPI-stimulated serum pepsinogen A in patients, suspected of having atrophic body gastritis, can be used as a non-invasive marker of the presence and severity of mucosal corpus atrophy. An abnormally low serum pesinogen A respons to PPI-administration supports strongly the diagnosis of severe body atrophy.

The 14-day course was selected because it is the period required to achieve highest levels of pepsinogen A in a study in 8 healthy volunteers with omeprazole 30 mg daily by Festen e.a. [22].

The mechanism of the effect of PPIs on serum pepsinogens remains speculative. Omeprazole [36] and somatostatin [37] inhibit pepsinogen-secretion into the gastric lumen. In vitro experiments with isolated gastric glands show that the sum of exocrine and endocrine secretion of pepsinogen A increases after omeprazole [38]. The combination of these data suggests that inhibition of exocrine secretion followed by increased storage and release of pepsinogen A into the circulation is probably responsible for the effect of omeprazole on serum pepsinogen A levels.

There are no data on the biochemical effect of PPIs on serum pepsinogens in gastric atrophy.

It is obvious that with the progressive loss of specialized chief cells in the oxynic mucosa and the parallel diminishing production of the pepsinogens, there is no storage of pepsinogen A for exocrine secretion and to release into the circulation, when gastric secretion is inhibited.

The mechanism of the effect of PPIs on serum gastrin seems to be more natural. Intragastric acidity regulates the gastrin release from the antral G-cells through a negative feedback mechanism: when the gastric juice pH falls due to high parietal cell secretion, the release of gastrin is inhibited, whilst when the gastric juice pH rises, hypergastrinaemia ensues to stimulate the parietal cell mass. Gastrin, as a regulatory peptide, is secreted directly in the ciculation [39,40]. Therefore, it is comprehensible that gastric acid suppression increases circulating gastrin serum concentrations [41]. In humans, serum gastrin levels may increase up to four-fold when treated with PPI [41,42]. However, in patients with advanced antral mucosal atrophy with low number of G-cells, gastrin levels will be low and stimulation has less or no effect (patient nos. 16 and 25 in *table 1*).

The diagnosis of gastric atrophy is usually made by endoscopy with histological examination of biopsies. However, as to endoscopical and histological findings there is a well-known discrepancy between routine and "accurate" analysis of atrophic gastritis. A pre-endoscopical, reliable prediction of atrophy, a more appropriate biopsy plan and an informed skilled pathologist improve the identification of atrophy significantly and show that the prevalence of this condition is higher than usually

diagnosed [21]. It is in our opinion unacceptable that patients with preneoplastic conditions pass undiagnosed, even after an invasive diagnostic procedure.

In this context measuring basal pepsinogen and in borderline test results PPI-stimulated pepsinogen might well represent a good solution.

The capability of predicting pre-endoscopically the atrophic mucosal status would permit the application of an appropriate biopsy plan, with a sufficient number of biopsies, so as to limit the problem of the patchy distribution of the lesions and better characterize the histo-pathological aspects of conditions at risk of neoplastic degeneration. Furthermore, the endoscopist and, more particularly, the pathologist could be alerted about the possible presence of atrophic and other important disorders.

This study demonstrates that the PPI-atrophy test is a simple, accurate, and economic tool that allows refined detection of gastric atrophy and helps to determine the severity of atrophic gastritis. All this seems to imply that atrophic status could be assessed even in primary care setting and an optimal selective invasive diagnostic program could be achieved with cost reduction.

A limitation of our study is the relatively small number of patients studied, with, as a consequence, a low statistical power. However, the results of the present study demonstrate clearly the diagnostic potential of the PPI-stimulated pepsinogen in the diagnosis of gastric body atrophy. Further research in a larger group of patients has to give an answer on the question if the PPI-atrophy test deserves a place in the diagnostic field of gastric atrophy.

In *conclusion*, the present study shows that the pepsinogen respons to short term PPI-administration is abnormally low in patients with low basal pepsinogen concentration due to atrophy of the oxyntic mucosa. The PPI-stimulated increase of pepsinogen A has an inverse relation with the grade of atrophy. Therefore, the serum pepsinogen respons to PPI can be used as a marker to determine the severity of gastric corpus atrophy and to identify patients with body atrophy in case of borderline basal pepsinogen.

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Chapter VIII

◆

There are two ways of defining something,
firstly by saying what it is,
and secondly by saying what it is not.

◆

Chapter VIII

Role of Helicobacter pylori infection in the development of pernicious anaemia: a pilot study in primary care

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Keywords: Pernicious anaemia, *Helicobacter pylori*, Atrophic corpus gastritis, Primary care

Abstract

Background: Pernicious anaemia (PA) is associated with hypochlorhydric atrophic corpus gastritis and considered an organ-specific autoimmune disease. An important cause of chronic atrophic gastritis worldwide is *Helicobacter pylori*. The role of *H. pylori* in the development of autoimmune type of atrophic gastritis is unresolved.

Aim: To determine the frequency of *H. pylori* infection in the early stages of the severe corpus atrophy that is characteristic of overt PA which is usually *H. pylori* negative.

Design: Family pilot study.

Setting: Community-based primary health care setting in a rural area in the South-West of The Netherlands.

Patients & methods: The seroprevalence of *H. pylori* and parietal cell antibodies (PCAs) was studied in 50 first- and second-degree relatives of 8 PA-probands and in a control-group of 45 first- and second-degree relatives of 8 hypertension-probands. Hypertension (Ht) as control disorder is as a rule not associated with *H. pylori*. Additionally, serum atrophy markers, pepsinogen and gastrin, were measured to detect serological gastric body atrophy in both groups and questionnaires on gastric symptoms, past gastric diseases and autoimmune related comorbidity were collected and analysed.

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Results: 2 (25%) of 8 PA-probands and 3 (37.5%) of 8 Ht-probands were *H. pylori*-positive. We found 7 PA-relatives with circulating anti-*H. pylori* antibodies (14%) and 3 Ht-relatives (6%) (p= 0.3239, ns). We found serological gastric body atrophy in one, 28-years old, female patient of the PA-relative group (2%), who did not show evidence of *H. pylori*-infection; in the control group no patient with serological gastric body atrophy was documented. The prevalence of PCAs in the PA-relative group was 6% (3 subjects), vs 0% in the control group. One patient in the PA-relative group had both PCAs and antibodies to *H. pylori* (2%), whereas nobody in the control group showed this combination. Autoimmune-comorbidity was present in 6 PA-relatives and in none of the control group (p=0.0277, s).

Conclusion: This pilot study showed that *H. pylori* infection does not seem to be a decisive causative agent in the development of the gastritis characteristic of pernicious anaemia. In genetically predisposed subjects, the onset of authentic pernicious anaemia seems mainly related to autoimmune factors.

Introduction

Pernicious anaemia (PA) is a megaloblastic anaemia due to atrophy of the mucosa of the body of the stomach which, in turn, is brought about by autoimmune factors[1]. PA is the end stage of type A chronic atrophic autoimmune gastritis and is reported to occur with a frequency of 1.9% in Western populations over the age of 60 [1]. The gastritis results in the loss of parietal cells in the fundus and body of the stomach. Destruction of these cells curtails the production of intrinsic factor and subsequently limits vitamin B_{12} absorption. Laboratory evidence of parietal cell antibodies is approximately 85 to 90 percent sensitive for the diagnosis of PA. However, the presence of parietal cell antibodies is non-specific and occurs in other autoimmune disorders. Intrinsic factor antibody is only 50 percent sensitive, but it is far more specific for the diagnosis of PA[2].

PA was first described by Thomas Addison in 1849 and the disease was really "pernicious" because of the extreme type of anaemia with frequently lethal expiration[3]. Nowadays, PA is fully under control by treatment with parenteral or oral vitamine B_{12}, mostly in general practice[2].

Although the disease is silent until the end stage, the underlying gastric lesion can be predicted many years before the anaemia develops. The time span for progression of chronic atrophic gastritis due to gastric parietal cell antibodies to gastric atrophy and clinical anaemia is 20-30 years[1]. This condition leads to achlorhydria and intestinal metaplasia, which is a known risk factor for adenocarcinoma. A threefold increased risk for stomach cancer was observed in a population-based cohort study of almost 4600 patients with PA in the Uppsala Health Care Region of Sweden[4]. Both the inflammatory process, preceding PA, and PA *per se* may be factors leading to malignant transformation.

The pathophysiology of autoimmune gastritis is centered on the hydrogen-potassium ATPase gastric proton pump. This pump is present only in the parietal cells of the gastric body. The initial event appears to be a CD4+ T-cell reaction against the proton pump. The T cells cause parietal cell injury resulting in exposure of both intrinsic factor and the hydrogen-potassium ATPase to antigen-presenting cells. The immune response is activated with secretion of interferon and cytokines, and formation of antibodies against intrinsic factor and the gastric proton pump [1,5].

Although the immune respons is directed only against the components of the parietal cell, overtime there is destruction of both the chief and parietal cells [1,6]. Therefore, the loss of the zymogenic cells is probably secondary to the primary autoimmune reaction directed toward parietal cell H^+/K^+ -ATPase.

Autoimmunity is a complex process that likely results from inappropriate responses of the immune system to self antigens. Candidate aetiological factors include genetic abnormalities and infections. Several lines of direct and indirect evidence suggest that infectious agents may influence the occurrence or the course of some autoimmune diseases [7].

Helicobacter pylori (*H. pylori*) is a frequent gastrointestinal infectious agent, found world-wide [8-10]. Whether this infection is involved in the development of chronic atrophic gastritis leading to PA is unclear. Investigations on this issue in the eighties and nineties of the last century, based mainly on serological and histological findings, have given rise to different results. Some authors report a low prevalence of *H. pylori* infection, from 3 to 21%, in patients with PA [11-13]. Contrary observations have been made by others who report that PA-patients may have previously unrecognised high prevalence of *H. pylori* infection, showing a prevalence even up to 70% [14-16]. More recently, it is considered that various autoimmune and apoptotic sequelae induced by *H. pylori* appear to influence the pathophysiology of chronic gastritis thereby displaying histopathological and clinical features that are similar to those of autoimmune gastritis [17,18].

However, there are still many questions regarding the exact role of *H. pylori* in gastric autoimmunity and the pathogenetic mechanisms involved in the development of gastric corpus atrophy. The summation of multiple defective tolerance mechanisms may play a role [19]. It has been reported that the presence or absence of antibodies to *H. pylori* in PA patients cannot address aetiology alone [20]. *H. pylori*-related PA would, nevertheless, be expected to be associated with antral evidence of present or past infection, whereas in pure autoimmune PA, the antral mucosa would be normal [20].

Recently, an Italian study in a consecutive population of 81 PA patients showed that *H. pylori* could play a triggering role in a subgroup of PA patients [21]. Furthermore, another study in a population of 150 consecutive atrophic body gastritis patients demonstrated that 66% had evidence of *H. pylori* infection [10] and that *H. pylori*-associated gastritis is one of the routes by which patients arrive at the autoimmune endpoint.

When all this information is collated, it is very attractive to impute an initiating and promoting role to *H. pylori* in the development of extensive corpus mucosal atrophy with subsequent PA in some genetically susceptible individuals. Autoimmunity, in a form of autoantibodies, is common after many infections and may well result from the mimicking of host proteins by antigens of the infectious agent. There are, however, few if any examples in humans where molecular mimicry gives rise to autoimmune disease [22].

In the light of identifying the genetic, environmental and endogenous factors, responsible for the nature of PA, it is useful to study the pre-atrophic and the pre-pernicious anaemia stage of the disease. Although gastric autoantibodies are often found in adults with chronic *H. pylori* gastritis, *H. pylori* infected children have a low prevalence of gastric parietal cell antibodies [23,24]. It remains to be studied at what age and in which group of *H. pylori* infected individuals gastric autoantibodies appear.

Primary care research could possibly help to shed further light on the pathogenesis of PA, focusing on the pre-atrophic and pre-pernicious stage.

Previous PA studies reflect mainly the situation at the end-stage of the process [25,26]. Actually, prospective studies during the pre-pernicious stage of gastritis are needed but pragmatically not feasible. The pre-stages of autoimmune gastritis are poorly identified and certainly difficult to find in the general population. On the other hand, they might accumulate in PA-relatives, who show a distinct aggregation of end-stages of the gastritis process [27].

No studies, so far, have been done entirely in a primary care setting to date looking for association between *H. pylori* and the specific form of atrophic gastritis characteristic for PA.

A more thorough understanding of this possible association from a primary care perspective could be achieved by means of a family pilot study. Leading thought with the family study is the genetic predisposition to PA [28] and the theory about the intra-familial transmission of *H. pylori*. The epidemiological literature points out that the infection is acquired by oral ingestion of the bacterium and is mainly transmitted within families in early childhood [29,30].

For this reason we examined the seroprevalences of *H. pylori* infection in first- and second degree relatives of PA patients and compared the results with those obtained from first and second degree relatives of patients with hypertension, which is known to be as a rule not related to *H. pylori* infection. The prevalence of parietal cell antibodies (PCAs) was also determined in both groups.

In addition, we tested the PA- and Ht-relatives for gastric body atrophy, being the specific organ lesion, using the indicators pepsinogen A, pepsinogen C and gastrin. Finally questionnaires on gastric symptoms, past gastric diseases and autoimmune related comorbidity, were analysed.

The aims of the study were, then, to evaluate the behaviour of *H. pylori* infection in the two population series, and to determine latent PA and the autoimmune milieu,

considering low pepsinogen A concentrations and antibodies to parietal cells as selective markers for the diagnosis of clinically latent PA. Furthermore, we aimed to assess the prevalence of autoimmune co-morbidity in the PA and Ht-relative groups.

Methods

Setting

Subjects from the community-based family practice of one of us (AK) in 's-Gravenpolder, a rural village in the South-West part of The Netherlands, were invited to participate in this study. Informed consent was obtained from all participants in concurrence with the declaration of Helsinki.

Patients

Pernicious anaemia series

Patients who were receiving vitamin B_{12}- injections were identified using a computer-assisted search. Patients' records were then reviewed to determine reasons for the use of vitamin B_{12}-injections and the extent to which diagnostic criteria were established. All patients as having PA were diagnosed on the following criteria: initially low levels of serum vitamin B_{12} and macrocytic anaemia, the presence of parietal cell and/or intrinsic factor antibodies, the presence of atrophic body gastritis with histological confirmation of atrophy and response to vitamin B_{12} therapy. Ultimately, 8 probands (3 men and 5 women; mean age 74 years, range 66–80 years) with overt PA were selected. Their PA-relative family series consisted of 50 first and second-degree subjects (mean age 27 years, 27 F and 23 M, range 10–52 years). All subjects were asked to donate a fasting blood sample and to fill in a medical history related questionnaire.

Hypertension series

Eight control probands with known controlled hypertension (3 men and 5 women, mean age 70 years, range 61–82 years) were selected as a control group. Controlled hypertension was defined as diastolic blood pressure ≤ 90 mm Hg and systolic bloodpressure ≤ 160 mm Hg. Patients were selected using a computer-assisted search, matched from the general family practice by age and sex. Exclusion criteria were gastric resection and severe disabling diseases. The Ht-relative family series consisted of 45 first and second-degree subjects, 20 F and 25 M, mean age 30 years, range 10–54 years. All participants were also asked to donate a fasting blood sample and to fill in the abovementioned questionnaire.

Serology & hematology

Assessment of H. pylori infection

All serum samples were examined by using a validated enzyme immunoassay detecting specific immunoglobulin G against a homogenate of 6 strains of *H. pylori* and the results were expressed as the absorbance index (AI) of the sample versus a reference serum: serum with an AI > 0.32 IgG *H. pylori* antibody was considered positive [31].

Autoimmune serology

Parietal cell (PC) - and intrinsic factor (IF) auto-antibodies were analysed using commercial available kits, respectively for PC Autoscreen I, Scimedx Corporation, Denville, NJ 07834, USA and for IF Genesis Diagnostics Ltd, Little port, UK.

Serum biopsy

All serum samples were tested by well validated radio-immunoassays for levels of pepsinogen A (PgA), pepsinogen C (PgC) and gastrin [32]. Our validated criteria for advanced serological gastric body atrophy, corresponding to pentagastrine refractory achlorhydria or severe hypochlorhydria (PAO < 5 mmol/hr), expressed in the level of the serum markers, are a serum concentration of PgA < 17 μg/l, a PgA/C ratio < 1.6 and an accompanying serum concentration of gastrin > 100 ng/l [31-33].

Hematology

Serum vitamine B_{12} concentration was tested by Immulite 2000, DPC.

Hemoglobin level and mean corpuscular volume were measured with standardized tests.

Questionnaire

The questionnaire covered:

1. gastric symptoms, ulcer like, reflux like or atypical;
2. past ulcer disease and family history of ulcer disease;
3. drug use, smoking and alcohol consumption;
4. comorbidity in the form of autoimmune related disorders, like hyperthyroidism, diabetes, rheumatic arthritis, vitiligo and family history of autoimmune disease.

Statistical analysis

The Chi-square test was performed in calculations of differences between the two family series, considering a p-value of less than 0.05 statistically significant.

Results

H. pylori infection in probands and in PA- and Ht-relatives 2 (25%) of the 8 PA-probands were *H. pylori*-positive, and 3 (38%) of the 8 Ht-probands were *H. pylori*-positive.

Seven of 50 PA- relatives investigated, 5 women and 2 men, mean age 38 years (range 29-49), were found to have circulating antibodies to *H. pylori*.

Three of 45 Ht-relatives investigated, 3 men, mean age 36 years (range 17-47), were *H. pylori*- positive.

The overall prevalence of *H. pylori* infection in PA- and Ht- relatives, based upon the IgG score, was respectively 14% and 6%. This difference is considered to be not statistically significant (p = 0.3239). See also *table 1 and 2*

	PA-relatives n = 50	Ht-relatives n = 45
Men / women	27 / 23	20 / 25
Mean age in yrs (range)	27 (10-52)	30 (10-54)
H. pylori-antibodies	7 (14%	3 (6%)
Parietal cell-antibodies	3 (6%)	0
H. pylori & parietal cell antibodies	1 (6%)	0
Serological gastric body atrophy	1 (2%)	0
Autoimmune-comorbidity: (hyperthyroidy, diabetes, vitiligo, rheumatoid arthritis)	6 (12%) (p=0.0277)	0

Table 1. *Features of pernicious anaemia- and hypertension-relatives.*

Pernicious Anaemia Probands (N=8)					Pernicious Anaemia Relatives (N=50)			
Nr.	Age/sex yrs	Anti-PC/IF	AntiHp +ve	Concomitant AutoImmDis	TotalNr relatives	Anti-PC + ve	AntiHp +ve	Concomitant AutoImmDis
1.	74 / M	+ / +	–	–	9	1	1	4
2.	73 / M	– / +	–	–	4	1	0	0
3.	78 / F	+ / –	–	–	7	0	3	0
4.	66 / F	+ / –	+	–	8	1	0	2
5.	78 / F	+ / –	+	–	9	0	0	0
6.	80 / F	+ / –	–	+	6	0	0	0
7.	68 / M	+ / +	–	–	3	0	3	0
8.	77 / F	+ / –	–	+	4	0	0	0

Table 2. *Characteristics of 8 pernicious anaemia probands and their relatives*

Serological gastric body atrophy

In the PA relative population there was 1 (2%) young female patient of 28 years with a full blown profile of serological gastric body atrophy without complaints. She showed no evidence of *H. pylori* infection, (IgG 0.10).

In the Ht-relative group no patients with serological gastric body atrophy were found.

Parietal cell antibodies (PCAs) (see table 3)

Three female patients (6%) of the 50 PA-relatives, mean age 28 years (range 10-47) had PCAs; 1 person (46 year) of the 3 had also *H. pylori* antibodies.

In none of the Ht-relatives PCAs were found.

	Sex	Age yrs	Hpylori ve / IgG	Gastrin (ng/l)	PgA (Ìg/l)	Ratio Pg A/C	Hb mmol/l	MCV
1	F	28	– / 0.10	3300	4	0.2	8.2	86
2	F	46	+ / 0.96	120	53	0.9	8.5	85
3	F	10	– / 0.06	19	30	3.1	8.5	88

Table 3. *Characteristics of 3 PA-relatives with antibodies to parietal cells*

Full blood count and vitamine B_{12} levels

Neither the PA-relatives, nor the Ht-relatives had macrocytic anaemia and low vitamine B_{12} levels.

Questionnaires

Six of the PA-relatives, among them 2 of the 3 subjects with PCAs, did report autoimmune comorbidity, to wit 4 hyperthyroidism, 1 diabetes and vitiligo and 1 rheumatoid arthritis.

Nobody in the control group did show autoimmune comorbidity (p=0.0277,s).

Of the 7 PA-relatives with *H. pylori* seropositivity 1 person had never stomach complaints, 5 seldom and 1 sometimes. Also in the 43 *H. pylori* negative persons there were no relevant stomach complaints.

Of the 3 Ht-relatives with *H. pylori* seropositivity the youngest of 17 years never had stomach problems, whereas the other 2 men had sometimes stomach complaints. The 42 *H. pylori* negative Ht-relatives did not have gastric complaints.

Nobody of the PA- ad Ht-relatives had a history of ulcer disease, neither an ulcer-positive family history. No statistically significant differences were detected between the 2 groups as regards alcohol consumption, coffee intake and smoking habits.

Discussion

Autoimmune diseases affect about 5% of individuals in developed countries [34]. Epidemiological investigation in the USA in 1997 showed a 3-rd place of pernicious anaemia (PA) in the top 6 prevalence rate of autoimmune diseases [35], so every care-providing physician has several overt and prospective PA-patients in his or her family practice.

Our study was inspired by the common opinion that autoimmune disorders require environmental triggers superimposed on a genetic predisposition and that of the many potential environmental factors, infections are the most likely cause. So the link to *H. pylori* in the pathogenesis of PA might be obvious.

Already in the 90s of the last century several studies have been performed on the association between *H. pylori* infection and gastric autoimmunity [25,26]. In the last few years there is also interest in a possible link between *H. pylori* infection and a miscellany of extragastric disorders which also include autoimmune diseases [36-38]. Nowadays, it is little by little the prevailing view that *H. pylori* is an essential component of gastric autoimmune disease; thus the host and environmental factors are easily ignored [18,21,39].

What are the facts? Patients with PA (type A gastritis) develop atrophic gastritis mainly in the fundus and corpus. In contrast, patients with *H. pylori* infection (type B gastritis) develop atrophic gastritis in the antrum that over 15 to 20 years may progress to involve the entire stomach, usually occurring without an autoimmune component. Atrophic gastritis of the fundus with an autoimmune component, which may eventually lead to PA after age 55 [40], is more frequently seen in women than in men [41]. This phenomenon is not unique since it is well known that females produce more vigorous cellular and humoral immune reactions and have autoimmune diseases more frequently than males [42,43]. In contrast, atrophic gastritis is most commonly seen in men with *H. pylori* infection [44].

Other evidence against the role of *H. pylori* infection in PA is that in countries with a high prevalence of *H. pylori* and a high prevalence of atrophic gastritis, PA appears to be uncommon [45]. Most of the published studies indicate that patients with PA are infected with *H. pylori* less often than are age-matched controls [26,46-48].

These findings seem in apparent contrast with the hypothesis that *H. pylori* infection may be linked to the onset of PA.

Anyhow, several indirect arguments support the idea that microbial agents influence the occurrence or course of certain autoimmune diseases. The mechanism of action remains unclear. The concept of a cross mimicry mechanism with immunological cross-reactivity between anti-pathogen and anti-self responses, is a plausible explanation to elucidate the association between infectious agents and autoimmunity [6]. In particular, it has been assumed that some infectious agents may express some antigens which are related to specific self-components. In the last decade,

some studies have described that the presence of anti-gastric antibodies induced by *H. pylori* infection was due to a cross mimicry between Lewis blood group antigens expressed both by the protonic pump of gastric epithelium and the bacterium itself[17,35].

According to the line of indirect evidence, it might be plausible that *H. pylori* could have occurred in the early phases of autoimmune gastritis, for example in asymptomatic parietal cell-antibody (PCA)-positive subjects[48], and that the progressive decrease in acid secretion hampers bacterium survival, which undergoes spontaneous eradication with time in the hostile atrophic environment. This theory is supported by a 32-year longitudinal study which reported the appearance of PCAs and chronic autoimmune gastritis in 6 persons, 5 of them were *H. pylori*-positive at first examination. Nevertheless, with the appearance of severe grades of corpus atrophy, all initially infected patients became *H. pylori* negative along with a normalization only of antral inflammation[49].

So, in trying to clarify the putative role of *H. pylori* infection in PA, it is desirable to identify the pre-pernicious anaemia stage of the so called type A gastritis and to undertake prospective studies[25].

In an attempt to approach this goal we studied the prevalence of *H. pylori* infection, the biochemical markers of latent PA and the autoimmune milieu in a population of close PA-relatives, i.e children and grandchildren, considering low serum pepsinogen A and antibodies to parietal cells as selective parameters for the diagnosis of clinically latent PA. The control group of close Ht-relatives underwent the same examination.

Our study does not show a significant difference in *H. pylori* prevalence between PA-first and second-degree relatives and Ht-first and second-degree relatives as a control group (14% vs 6%, $p<=0.3239$).

It is noteworthy that the seroprevalence of H. pylori of 14% in the PA-relative group with a mean age of 27 years with also a few children (≥ 10 years) is relatively high. The seroprevalence of H. pylori of 6% in the Ht-relative group seems compatible with the age-related prevalence in the general population.

One PA-relative person, age 46 years, with *H. pylori* positive serology had also antibodies to parietal cells, possibly as an epiphenomenon or a consequence of *H. pylori* gastritis as stated by Negrini[17].

In the present study, the prevalence rate of parietal cell antibodies was significantly higher in the PA-relatives than in the Ht-relative group (6% vs 0%). Owing to the small number of PCA-positive subjects, the statistical power of the prevalence rate is low. Remarkable is the young age of 2 female patients, respectively 10 and 28 years, and the negativity of *H. pylori* serology. The third female person, age 46, is also positive to *H. pylori* infection.

The female PA-relative person, age 28 years, appeared to have a clear cut hypopepsinogenaemia and hypergastrinaemia, compatible with gastric body atrophy. She showed negative *H. pylori* serology. She also suffered from Graves'disease. The pathogenesis of autoimmune atrophic body gastritis in young persons seems fully determined by genetic and immunological phenomena whereas microbiological factors are not decisive [50]. It is remarkable that the mother of this young female person was clearly *H. pylori* positive as PA-proband.

In the control group no persons with hypopepsinogenaemia were found.

Furthermore, taking into account that PA and the predisposition to PA may be part of a autoimmune polyendocrine syndrome, we were interested in the history of the PA-probands and the relatives concerning autoimmune-related comorbidity. Six persons (12%) of the PA-relative group mentioned autoimmune comorbidity *(table 1 and 2)* and nobody in the control group (p=0.02777,s). One patient of the 6 PA-relatives with autoimmune comorbidity appeared to be *H. pylori* positive and 2 patients of the 6 had anti-PCAs *(table 2)*.

The data of two PA-probands (25%) showed autoimmune comorbidity.
It is noteworthy that none of the *H. pylori*-positive patients had stomach complaints.

The main limitation of the present study is the relatively small number of patients studied, without a statistical power calculation. This might be a reason for the lack of siginificant differences between the study and the control group. Another limitation is that we do not have gastric biopsies and histopathology of the two investigated groups. So the histological prevalence of gastric atrophy in both groups could not be assessed. Endoscopical examination was not done because of ethical objections. Population-based studies preclude the biases associated with health-seeking that affect clinic-based studies. However, population-based studies can be limited by the failure to use valid instruments for measurements of diseases.

Nevertheless, the present article overviews the complex causes of autoimmune gastritis, providing insights on the background of triggering factors in the onset of gastric autoimmunity, regarding a homogenous cohort of subjects. It can give a meaningful aid to the end-users in clinical practice, that is the general practitioner.

In summary, despite the plausible seroepidemiological association of *H. pylori* infection with autoimmune gastric body atrophy, suggested in several recently published studies [17,18,21] our data did not show such an association for the so-called latent PA-patients. Our results provide some arguments for a mainly autoimmune oriented pathogenesis of PA, to wit: the non-significant difference in *H. pylori* prevalence in the PA-relative group compared with the Ht-relative group, furthermore the case finding of a young latent PA-patient of 28 years without *H. pylori* infection and finally the significant difference in autoimmune comorbidity between the PA-relatives and the control group of Ht-relatives.

The role of *H. pylori* infection in the pathogenesis of PA is probably only one,

not decisive, factor in the spectrum of mechanisms producing autoimmune gastritis [51]. Autoimmune diseases present a challenge for medical research to unravel the multiple mechanisms of tissue injury. Primary care research should not shrink from playing a role in this unraveling process. Further studies in primary care preferably on a larger scale should be undertaken to define more precisely our observed results.

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Chapter IX

◆

Circumstancial evidence:
better roughly right than precisely wrong.

◆

Chapter IX

Summary, comments and final remarks

Currently, there is a large body of information regarding the diagnostic significance of the gastric biomarkers. Gastric serological methods are objective and convenient, and non-invasive methods are superior for repeated analyses. Worldwide experience with gastric serum profile underlines its added value, especially in the evaluation of gastric inflammation.

It is remarkable that there have been hitherto no studies at the primary care level using gastric serology. Such information is needed, as this may have impact on patient care.

Research conducted in general practice is potentially valuable because the results do reflect the situation in the general population. The study population is in the nature of things unselected. Population-based studies in primary care setting preclude the biases associated with health-seeking that affect clinic-based studies. This thesis enlightens for the first time the serum profile of gastric mucosa fully in primary care setting.

The research in this thesis is focusing on gastritis with gland loss. An important sequel of gland loss is the deterioration of gastric secretory function. When atrophy affects a large part of the stomach, it is associated with acid hyposecretion, impaired pepsinogen levels and hypergastrinaemia. Recent research points to accurate corresponding of serological gastric profile with the histological morphometric diagnosis of atrophy.

The gastric serum profile provides three important advantages: *1.* overall evaluation of the gastric mucosa, *2.* non-invasiveness and *3.* low costs.

These characteristics make the gastric serum profile suitable for seroprevalence studies in large populations. In general practice the gastric serum biopsy can be used as a first approach in the "test and scope" and/or "test and treat" strategy in the management and risk counselling of subjects at risk for gastric cancer, i.e. first and second degree relatives of patients with non-cardia gastric cancer.

In ***Chapter 1*** an overview in depth is presented of current knowledge about the biological background of the gastric serum biopsy. Its non-invasive diagnostic potential is described with focus on the serum profile of atrophic gastritis. Atrophic gastritis is considered to be a preneoplastic lesion and therefore efficient detection of atrophic gastritis is an important public health issue. Current knowledge about aetiology and histology of atrophic gastritis is enlightened. The serological gastric biopsy can serve as diagnostic test for atrophic gastritis with regard to accuracy and conveniency, together with low burden and costs. This detection can be performed in primary care setting and should be restricted within high risk groups such as family members of patients with gastric cancer.

Chapter 2 presents the character, aims and outline of the thesis.

In ***Chapter 3*** the clinical usefulness of the serological gastric biopsy in the management of dyspeptic patients in primary care is addressed, based on a survey of literature. Primary care physicians must resist their own or patients pressure to a hurry up-referral for endoscopy, and they should always wonder greatly what would be the diagnostic outcome of gastroscopy. In daily practice, the most important gastric organic diseases are the various types of gastritis (chronic superficial and chronic atrophic), peptic ulcers and rarely gastric cancer. Actually, the main motive of a primary care physician for an endoscopy referral is the fear of missing gastric cancer. The most common cause of gastritis is *H. pylori* colonization. Inflammation and atrophy of the gastric mucosa result in impairment of the gastric secretory functions. Patients with atrophic chronic gastritis can serologically be distinguished from those with non-atrophic chronic gastritis and from those with normal mucosa, respectively. The serum profile of gastric mucosa can be used to select patients for gastroscopy.

It is recommended that the general practitioner should take knowledge of the gastric serum biopsy and that he/she should give it a relevant place in his/her way of thinking about gastric diseases.

Comments

Although the serological gastric biopsy has been accepted as a valuable diagnostic tool in clinical medicine, little is known about its value for the primary care.

For acceptance and application of the gastric serum biopsy in daily practice, randomised clinical trials in primary care are needed for evaluation of sensitivity and specificity and outlining of strategy.

In ***Chapter 4*** the prevalence of chronic atrophic corpus gastritis in a large primary cohort of 997 subjects in the Dutch province Zeeland was evaluated by means of serology, i.e. serum pepsinogens A and C, and gastrin. Additionally, the two main causes for the promotion of atrophic gastritis, *H. pylori* infection and autoimmune factors were addressed in the serological atrophy group and in an age and sex matched nested control group without serological evidence of atrophy. As such, the seroprevalence of serological advanced atrophic corpus gastritis in this primary community was found to be 3.4%. Although the two main causes, *H. pylori* infection and autoimmunity, work synergistically in the promotion of atrophic gastritis, most atrophic gastritis is thought to be induced by *H. pylori* infection. However, in this primary care study when compared to nested case controls, the odds ratio of having atrophic corpus gastritis was surprisingly significantly higher ($p<0.025$) for parietal cell antibodies [24] than for *H. pylori* antibodies [1.62]. In view of the rapid decreasing risk of *H. pylori* infection in the Western world it is likely that the impact of *H. pylori* on the development of atrophic corpus gastritis will further diminish.

Comments

This explorative study aimed to assess the prevalence of chronic atrophic gastritis as precursor lesion of gastric cancer in a general practice in Zeeland, using the "Samloff " gastric serum biopsy as diagnostic instrument

It is remarkable that the prevalence of gastric cancer in the Dutch province Zeeland is relatively high compared to the rest of the country. This higher prevalence is signalized by the NCR (Netherlands Cancer Registry) from the mid-1980's but this epidemiological phenomenon has never been investigated in general practice.

The seroprevalence of 3.4%, found in this study is relatively high compared to prevalences in other parts of the Netherlands. Schlemper et al. reported a prevalence of 1.6% in Dutch employees of companies in the Hague and Eindhoven in 1995. Bins et al. arrived at a prevalence of 2.4% in Dutch factory workers and their spouses in Arnhem in 1984. This differences in seropevalence is partially due to the difference in age of the study populations with elder subjects in our study population. However, it seems reasonable to assume that the higher seroprevalence of atrophic gastritis found in Zeeland, reflects the higher prevalence of gastric cancer in this part of the Netherlands.

H. pylori is regarded as playing a specific role in the development of atrophic gastritis.

However, the results of our present study indicate that the existence of positive anti-parietal cell antibodies correlates with serological atrophic gastritis of the corpus. Anti-parietal cell antibodies and serum gastrin are both specific biomarkers for gastric inflammation in the gastric corpus. On the basis of data in literature it seems that H. pylori antibodies are a significant risk factor for atrophy of the gastric antrum.

Gastric atrophy in the gastric corpus is a major target to evaluate the risk for gastric cancer.

Markers predicting gastric atrophy enable earlier identification of these risk patients.

Theoretically, the higher prevalence of atrophic body gastritis in Zeeland might be induced by family-linked concomitant hereditary factors in a relatively homogenic and stable population with large families and a high birth number. In this way the expression of autoimmune-associated conditions is more likely to become manifest.

It is noteworthy that in the above-mentioned study by Schlemper et al., evaluating the seroepidemiology of gastritis in Japanese and Dutch working populations, the seroprevalence in the Japanese group was 4.4% compared to 1.6% in the Dutch group, reflecting a higher prevalence of mild and severe mucosal atrophy of the corpus in the Japanese, apart from that among both H. pylori positives and negatives. Thus, these findings suggest that in the Japanese the development of atrophic gastritis is in part unrelated to H. pylori. Our seroepidemiological findings in the Dutch province Zeeland could be less or more compared with the findings in the Japanese group, ethnically prone to higher gastric cancer risk.

The explorative study in ***Chapter 5*** has investigated at the primary care level the utility of serological detection for atrophic body gastritis as a tool for selecting patients for endoscopy and gastric biopsies. Serological evidence of gastric body atrophy was assessed in a large population (997) of consecutive adults. A total of 34 subjects had evidence of serological atrophy and this evidence was persistent on retesting in 20 of 25 retested subjects.

These 25 subjects underwent endoscopy with gastric biopsy collection and all 20 who were positive on serological retesting showed histological evidence of gastric body atrophy. Further investigations revealed a mixture of *H. pylori* and autoimmune gastritis as aetiological factors. The results indicate that the combination of hypergastrinaemia, hypopepsinogenaemia and a low pepsinogen A/C ratio is a good serological marker for gastric body atrophy and correlates with the findings in gastric biopsies.

Comments

The study reveals a high positive predictive value for positive serological testing and the presence of gastric body atrophy. However, one patient with a false negative result shows that the serological profile can be influenced by gastric pathology outside the gastric body and it should therefore be interpreted with caution.

The lack of a control group is problematic, and this issue is alluded in the discussion. The key question is how many subjects with entirely negative serology also may have gastric atrophy. This question can only be answered by histological examination of many subjects with negative serology, which is ethically non acceptable. The 95% concordance in this study between serological and histological gastric body atrophy does suggest that the prevalence of unsuspected atrophy (if any) must be low.

Chapter 6 addresses the clinical utility of the ^{13}Carbon-urea breath test in identifi-

cation of *H. pylori* infection in asymptomatic patients with atrophic body gastritis. There is presently no doubt that infection with *H. pylori* is a major cause of chronic atrophic gastritis. A reliable detection of current *H. pylori* infection is important to take the decision to eradicate.

Because there is doubt about the diagnostic accuracy of ^{13}C-UBT in atrophic body gastritis this study tried to obtain a ruling on its reliability. We studied at the primary care level the performance of ^{13}C-UBT in 20 asymptomatic patients with histologically proven atrophic body gastritis and compared the results with culture as reference test. We compared the results also with serology. The overall accuracy of ^{13}C-UBT with culture appeared to be 85.0 % and anti *H. pylori* serology with culture showed an overall accuracy of 75.0 %. It is concluded that ^{13}C-UBT can be used as diagnostic *H. pylori* test in asymptomatic patients with atrophic body gastritis, preferably in addition to serology, to select subjects for anti-*H. pylori* therapy.

The diagnostic approach of the ^{13}C-UBT in *H. pylori*-positive patients with gastric atrophy probably also applies to patients with acid-peptic disease using antisecretory agents such as proton pump inhibitors or H_2-receptor antagonists.

Comments

The ^{13}C-urea breath test (UBT) is considered to be one of the most accurate ways of diagnosing ongoing Helicobacter pylori infection in non-atrophic gastritis. However, in atrophic gastritis documentation of H. pylori infection is difficult because of progressive disappearance of the bacterium. Values are clearly affected by decreased H. pylori bacterial load as a consequence of unfavourable atrophic circumstances. The only sign indicating a previous infection may be serology which, albeit, is not able to discriminate between present and past infection.

Remarkable is that our study at the primary care level has demonstrated, for the first time, a good performance of the ^{13}C-urea breath test as an active H. pylori diagnostic test in patients with subclinical atrophic body gastritis, this in clear contrast with previous studies with selected patients. Our explanation for this difference is our non-selected, asymptomatic, naïve primary care level population with verified (GP-controlled) no history of H. pylori eradication therapy and no history of medication with antibiotics the last 3 years. Evidence from patients selected by referral cannot easily be generalised to patients seen in primary care.

It is noteworthy that, in theory, the ^{13}C-UBT-results of this primary care study might be applicable in patients with artificial hypo- or achlorhydria using acid-suppressive therapy, this in partial contrast with data in literature.

Further studies on a larger group of subjects with atrophic gastritis in primary care are desirable for determining the optimal approach to diagnose H. pylori infection in such persons.

Chapter 7 reports on the results of a novel test measuring proton pump inhibitor (PPI)-stimulated serum pepsinogen responses in 25 patients with serological gas-

tric body gastritis. The results are compared with histological evaluation of corpus biopsy specimens in the same group of patients. The present methods to determine the severity of atrophic gastritis are hampered for various reasons. Gastroscopy with biopsies have the drawback of sampling errors, basal pepsinogen the disadvantage of fluctuations and pentagastrin stimulation of acid secretion the problem of low patient acceptance. The purpose of this study was to determine whether a decreased serum pepsinogen respons to short-term PPI-administration, indicating an impaired oxyntic chief cell function, can be used to determine the severity of atrophic body gastritis and possibly the increased risk for gastric cancer.

It is concluded that the serum pepsinogen A response to PPI is abnormally low in patients with atrophy of the gastric body. Our study evidenced clearly that PPI-stimulated increase of pepsinogen A in patients with atrophic corpus gastritis has an inverse graded relation with the severity of atrophy, in other words, the more advanced the atrophy, the less the serum increase of pepsinogen A. The characteristics reported here, together with its high accuracy, make the PPI-atrophy test an effective tool to determine the severity of atrophic body gastritis and to identify patients with body atrophy in case of borderline basal pepsinogen.

Comments

The principle of stimulation of function is a well-known diagnostic tool in medical investigation and research. It reveals most accurately loss of functional cell capacity.

The present study confirms the hypothesis that a decreased pepsinogen A respons to short-term PPI administration is a marker of the severity of gastric body atrophy.

PPI-stimulated pepsinogen might be a substitute for non-stimulated pepsinogen as marker for serological atrophic body gastritis. Further research should concentrate on test functionality and whether the PPI-atrophy test deserves a place as a complementary test in the grading of atrophic gastritis of the oxyntic mucosa as a risky condition.

The pilot study in ***Chapter 8*** aimed to evaluate whether *H. pylori* could be involved in the early stages of the severe corpus atrophy that is characteristic of overt pernicious anaemia and is usually *H. pylori* negative.

We addressed this question by examining the pre-atrophic and pre-pernicious stage of autoimmune gastritis. The pre-stages of autoimmune gastritis could be find in first and second degree relatives of pernicious anaemia patients. The seroprevalence of *H. pylori* infection and atrophic body gastritis was studied in 50 first and second degree relatives of 8 pernicious anaemia patients in our general practice and was compared with the same tests, obtained from 45 first and second degree relatives of 8 patients with hypertension, a disorder, which is known to be as a rule not related to *H. pylori* infection.

We found no significant difference in *H. pylori* prevalence between relatives of patients with pernicious anaemia and relatives of patients with hypertension as a control group, suggesting that *H. pylori* is not involved in the early pernicious anaemia

stages that lead to severe corpus atrophy. Genetic contributions to gastritis, whether due to autoimmunity or to *H. pylori* still await concluding chapters.

Comments

The knowledge about the essential nature of pernicious anaemia shows still gaps, two of which are singled out for comments.

First, the questionable role, if any, of infection with H. pylori in the initiation and perpetuation of autoimmune gastritis. Our primary care pilot study does not show a significant involvement of H. pylori but an important limitation of the study is the small number of patients, which reduces statistical power. Epidemiological data in the literature suggest that H. pylori can be present in the earlier stages of autoimmune gastritis. A long-term follow-up study of any material with accumulation of signs of autoimmune gastritis would be desirable.

Second, the unknown genes responsible for inherited predisposition to human gastritis. Pernicious anaemia seems ideal for informative studies on large family clusters, and the ensuing data could be applicable to autoimmunity in general.

The role of primary care research in this can only be very modest.

Final remarks

Currently, there is no compelling evidence that early detection of gastric atrophy, as precursor lesion of gastric cancer, reduces disease-specific mortality and improves quality of life. The research described in this thesis may be relevant to our evolving understanding of chronic atrophic gastritis as a multifactorial process. The host genetic factors set up the baseline immune respons to the *H. pylori* infection. Additionally, the environmental factors might play a major role in the modulation of this respons and the final determination of disease outcome. The effect of *H. pylori* treatment on prevention of gastric cancer development in chronic carriers is still unknown. Continuing research on this important health issue is warranted.

No calculation of sample size and statistical power was used in the studies of this thesis, so it is too early to draw conclusions about usefulness of gastric serum profile in general practice. The explorative studies were fully initiated and performed in the author's own practice. The favourable position of the general practitioner in the current Dutch health care system should be mentioned, making him a very successful promotor of compliance in his patients for participation in primary care research. The suggestion is put forward that the explorative studies in this thesis have gathered evidence to advocate the use of gastric serum profile in primary care. The studies are helpful to understand the complex mechanisms of gastric pathophysiology. In addition, this thesis provides further insight into a more underpinned diagnostic strategy considering gastric diseases.

There is a growing recognition of the need for rigorous research in primary care, including randomized controlled trials. Sufficient recruitment rates of both professionals and patients to studies are important because the findings of these studies are more likely to be generalizable. Current or aspiring future investigators should corroborate the results of this thesis, using a strong trial organization. This will not only satisfy scientific hunger, but will give the possibility to the general practitioner to choose the appropriate diagnostic methods in the field of primary care gastroenterology.

Samenvatting, commentaar en afsluitende opmerkingen

Er is vandaag de dag een grote hoeveelheid informatie beschikbaar over de diagnostische betekenis van het meten van serumspiegels van pepsinogeen en gastrine voor de kwalificering van de toestand van de maag. De bepaling van deze biomarkers is een objectieve, patiënt-vriendelijk meting, en als niet-invasief onderzoek ideaal voor herhaald onderzoek. Wereldwijde ervaring met het serumprofiel van de maag onderstreept haar toegevoegde waarde, in het bijzonder bij de evaluatie van ontsteking van het maagslijmvlies.

Het is opmerkelijk dat er tot nu toe geen eerstelijnsstudies zijn verricht die gebruik hebben gemaakt van de diagnostische waarde van maagserologisch onderzoek. Dergelijke studies zijn nodig omdat die van belang kunnen zijn voor de directe patiëntenzorg. Wetenschappelijk onderzoek door de huisarts is van potentiële waarde omdat de resultaten de gezondheidstoestand van de algemene bevolking weerspiegelen. De studiepopulatie is in de aard der zaak ongeselecteerd en onderzoek in de algemene praktijk sluit vertekening uit die altijd optreedt bij onderzoek van patiënten die verwezen zijn naar de tweedelijn.

Dit proefschrift beschrijft voor de eerste maal een aantal maagserologische studies die volledig verricht zijn in de huisartspraktijk.

Het wetenschappelijk onderzoek in dit proefschrift richt zich op gastritis en atrofie van de maagmucosa. Een belangrijk gevolg van verlies aan glandulair epitheel is het verslechteren van de secernerende functie van de maag. Als atrofie een groot deel van de maag treft, gaat dit gepaard met teruggang in zuurproductie, met verminderde serumpepsinogeen-spiegels en verhoogd serumgastrine. Recent weten-

schappelijk onderzoek toont aan dat het serologisch maagprofiel nauwkeurig correleert met de histologisch morfometrische diagnose van atrofie. Er zijn drie belangrijke voordelen te noemen bij de bepaling van het serumprofiel van de maag: *1.* de hele maag wordt onderzocht, *2.* de bepaling is niet-invasief, *3.* de serumbiopsie is goedkoop.

Deze eigenschappen maken de serumbiopsie van de maag bruikbaar voor populatiestudies. In de algemene praktijk kan de serumbiopsie gebruikt worden als een eerste aanpak met "testen en scopiëren" en/of " testen en behandelen" bij onderzoek en behandelen van en risico-voorlichting geven aan personen met een verhoogd risico op maagkanker, nl. de eerste en tweede graads familieleden van patiënten met maagkanker.

Hoofdstuk 1 geeft een uitgebreid overzicht van de huidige kennis van de biologische achtergrond van de serumbiopsie van de maag. De diagnostische betekenis van de serumbiopsie wordt beschreven, met speciale aandacht voor het serumprofiel van atrofische gastritis. Atrofische gastritis kan men zien als een precancereuze aandoening. Zodoende is efficiënte diagnostisering uit oogpunt van volksgezondheid belangrijk. Er wordt ingegaan op de huidige kennis omtrent oorzaken van atrofische gastritis en op de histopathologische aspecten. De serumbiopsie van de maag is een bruikbare detectietest voor atrofische gastritis omdat deze test betrouwbaar, patiëntvriendelijk en goedkoop is. Deze detectie kan verricht worden in de eerstelijnsgezondheidszorg en met name bij personen met een verhoogd risico op maagkanker, zoals familieleden van patiënten met maagkanker.

In ***Hoofdstuk 2*** wordt het karakter en de structuur van het proefschrift besproken samen met het doel van de verschillende exploratieve studies.

Hoofdstuk 3 beschrijft aan de hand van een overzicht van de literatuur de diagnostische betekenis van de serologische maagbiopsie bij de benadering van patiënten met dyspepsie in de huisartspraktijk. Huisartsen moeten hun eigen drang en die van hun patiënten bedwingen om niet al te snel te besluiten tot een endoscopie. Ze moeten zich altijd afvragen wat de diagnostische opbrengst zou kunnen zijn van de endoscopie. In de dagelijkse praktijk zijn de meest voorkomende organische maagafwijkingen de verschillende vormen van gastritis (chronisch superficieel en chronisch atrofisch), peptische ulcera en zelden maagkanker. Eigenlijk is de belangrijkste reden van de huisarts om te verwijzen voor gastroscopie de angst om maagkanker te missen. De meest voorkomende oorzaak van gastritis is infectie met *Helicobacter pylori.* Ontsteking en atrofie van de maagmucosa resulteren in een verstoring van de secretoire functies van de maag. Patiënten met atrofische gastritis kunnen serologisch onderscheiden worden van patiënten, respectievelijk met niet-atrofische chronische gastritis of met een normale mucosa. Zodoende kan de serumbiopsie van de maag gebruikt worden om patiënten te selecteren voor endoscopie.

Het wordt aangeraden dat de huisarts kennis neemt van de diagnostische betekenis van de serumbiopsie van de maag en dat hij/zij deze een relevante plaats geeft in de manier van denken over maagaandoeningen.

Commentaar
Hoewel de serumbiopsie van de maag beschouwd wordt als een waardevol diagnostisch instrument in de klinische geneeskunde, is er nog maar weinig bekend omtrent haar waarde voor de huisartspraktijk. Voor algemene erkenning en toepassing van de serumbiopsie van de maag in de dagelijkse praktijk zijn er gerandomiseerde trials nodig in de eerstelijn om de sensitiviteit en specificiteit vast te stellen en om een wijze van aanpak te definiëren .

In ***Hoofdstuk 4*** wordt de seroprevalentie van chronisch atrofische corpusgastritis nagegaan in een groot eerstelijnscohort van 997 personen in de Nederlandse provincie Zeeland met behulp van maagserologie, d.w.z met bepaling van serumpepsinogeen A en C en serumgastrine. In de gevonden serologische atrofiegroep en in een aselecte en in aantal gelijke controlegroep van patiënten zonder maagatrofie met gelijk geslacht en leeftijd wordt voorts aandacht besteed aan de twee belangrijke oorzaken van het bevorderen van atrofische gastritis, namelijk *H. pylori*-infectie en auto-immuniteit. De prevalentie van serologische gevorderde atrofische corpusgastritis in deze eerstelijnspopulatie blijkt 3.4% te zijn. Hoewel de twee hoofdoorzaken, *H. pylori* en auto-immuniteit samen de ontwikkeling van atrofische gastritis bevorderen, wordt aan *H. pylori*-infectie in het algemeen de grootste causale atrofiërende rol toebedeeld. Echter, in deze eerstelijnsstudie is verrassenderwijs, bij vergelijking van het infectiepercentage van de atrofiegroep met die van bovengenoemde gematchte controlegroep, de odds ratio om atrofische corpusgastritis te krijgen significant hoger ($p<0.025$) bij aanwezigheid van antistoffen tegen pariëtale cellen [24] dan bij aanwezigheid van *H. pylori*-antistoffen [1.62]. In het licht van het snel dalende risico van *H. pylori*-infectie in de Westerse wereld is het aannemelijk dat de invloed van *H. pylori* op de ontwikkeling van atrofische corpusgastritis verder zal afnemen.

Commentaar
Deze exploratieve studie behandelt de seroprevalentie van chronisch atrofische gastritis als precancereuze afwijking in een Zeeuwse huisartspraktijk, waarbij gebruik is gemaakt van de "Samloff" serumbiopsie van de maag als diagnostisch instrument.

Het is opmerkelijk dat de prevalentie van maagkanker in de provincie Zeeland relatief hoog scoort vergeleken met de rest van Nederland. Deze hogere prevalentie wordt door de NCR (Netherlands Cancer Registry) sedert het midden van de jaren 80 van de vorige eeuw gesignaleerd maar dit epidemiologische fenomeen is nooit onderzocht in de algemene praktijk.

De seroprevalentie van 3.4% voor atrofische corpusgastritis als precancereuze

afwijking die wij in deze studie vaststelden, is relatief hoog vergeleken met de prevalentie in andere delen van Nederland. Schlemper et al. beschreven een prevalentie van 1.6% bij Nederlandse werknemers van bedrijven in Den Haag en Eindhoven in 1995. Bins et al. kwamen tot een prevalentie van 2.4% bij Nederlandse fabrieksarbeiders en hun echtgenotes in Arnhem in 1984. Dit verschil in seroprevalentie is deels te wijten aan het verschil in leeftijd van de studiepopulaties waarbij onze populatie ouder is. Echter, het lijkt min of meer aannemelijk dat de hogere seroprevalentie van atrofische gastritis in Zeeland de hogere prevalentie van maagkanker weerspiegelt in dit deel van Nederland.

Algemeen wordt gedacht dat H. pylori een specifieke rol speelt bij de ontwikkeling van atrofische gastritis. Echter, de resultaten van onze huidige studie geven aan dat juist de aanwezigheid van antistoffen tegen pariëtale cellen correleert met serologische corpusatrofie van de maag. Antistoffen tegen pariëtale cellen en serum gastrine zijn beide specifieke biomarkers voor corpusgastritis. Op basis van literatuurgegevens schijnen antistoffen tegen H. pylori een significante risicofactor te zijn voor atrofie van het antrum.

Bij de evaluatie van het risico voor maagkanker is de opsporing van patiënten met corpusatrofie van de maag een belangrijk doel. Parameters die maagatrofie voorspellen maken vroege identificatie van deze risicopatiënten mogelijk.

Theoretisch gezien kan men stellen dat de hogere prevalentie van corpusatrofie in Zeeland geïnduceerd zou kunnen worden door familiegebonden, erfelijk gestuurde factoren in een relatief homogene en stabiele populatie met grote gezinnen en een hoog geboortecijfer. Op deze manier komen auto-immuun gerelateerde aandoeningen meer tot expressie.

Het is vermeldenswaardig dat in het bovengenoemd onderzoek van Schlemper, die de seroepidemiologie van atrofische gastritis bestudeerde bij een populatie van Japanse en Nederlandse werknemers, de seroprevalentie in de Japanse groep 4.4% was en in de Nederlandse groep 1.6%, hetgeen wijst op een hogere prevalentie van lichte en ernstige atrofie van het maagslijmvlies bij de Japanners, overigens zowel bij H. pylori positieve als bij H. pylori negatieve personen. Dus dit suggereert dat in een deel van de Japanse populatie atrofische gastritis is opgetreden zonder voorafgaande H. pylori infectie. Onze seroepidemiologische atrofie-bevindingen in de provincie Zeeland komen min of meer overeen met de bevindingen in de Japanse groep die qua ethniciteit een groter risico heeft om maagkanker te krijgen.

In ***Hoofdstuk 5*** wordt in een exploratieve studie onderzocht of in de huisartspraktijk serologisch onderzoek op atrofische corpusgastritis bruikbaar is als diagnostisch instrument om patiënten te selecteren voor endoscopie met biopsie. In een grote populatie van 997 opeenvolgende volwassen patiënten werd serologisch onderzoek verricht op maagatrofie. Er werden in beginsel 34 personen gevonden met atrofie en na serologisch hertesten werd bij 20 van 25 de atrofie bevestigd.

Deze 25 individuen ondergingen endoscopie met biopsie, en alle 20 die seroposi-

tief waren op atrofie toonden histologische corpusatrofie. Verder serologisch onderzoek liet een mengeling zien van *H. pylori*-infectie en auto-immuniteit als oorzakelijke factoren. De meeste personen hadden geen tot weinig klachten. De resultaten van dit onderzoek laten zien dat de combinatie van hypergastrinemie, hypopepsinogenemie en een lage pepsinogeen A/C ratio een goede serologische marker is voor atrofie van het corpusslijmvlies van de maag en dat deze marker goed correleert met de bevindingen in slijmvliesbiopten.

Commentaar

Deze studie laat een hoog-positieve voorspellende waarde zien van seropositief testen en de aanwezigheid van maagatrofie. Het fout-negatieve serologische profiel bij één van de patiënten geeft echter aan dat de serologische bevindingen kunnen worden beïnvloed door maagpathologie buiten het corpus en dat voorzichtigheid is geboden bij de interpretatie.

Een beperking van de studieopzet is het feit dat niet duidelijk is hoeveel personen met negatieve serologie ook maagatrofie zouden kunnen hebben. Daarom zou de negatieve voorspellende waarde uit deze controlegroep moeten komen. Dit gegeven kan alleen verkregen worden door een even grote groep met negatieve serologie ook te scopiëren en te biopteren. Wij vonden dit ethisch niet acceptabel. De 95% concordantie in deze studie tussen serologische en histologische corpusatrofie suggereert dat de prevalentie van onverwachte atrofie, als daar ooit sprake van is, laag moet zijn.

De belangrijkste reden voor de huisarts om maagserologisch onderzoek en H. pylori-eradicatie te overwegen, is het beantwoorden van de vraag van eerste en tweede graads familieleden van maagkankerpatiënten naar het risico wat zij lopen om ook maagkanker te krijgen.

Hoofdstuk 6 is een exploratieve studie naar de klinische bruikbaarheid van de ^{13}C-ureumademtest bij het aantonen van *H. pylori*-infectie bij asymptomatische patiënten met atrofische corpusgastritis. Er wordt vandaag de dag niet meer getwijfeld aan het feit dat *H. pylori*-infectie een hoofdoorzaak is van chronische atrofische gastritis. Het betrouwbaar vaststellen van een actieve *H. pylori*-infectie is belangrijk bij de besluitvorming om te eradiceren. Aangezien er twijfel bestaat over de diagnostische betrouwbaarheid van de ^{13}C-ureumademtest bij patiënten met atrofische corpusgastritis, hebben wij gemeend in deze studie deze betrouwbaarheid te toetsen. De resultaten van de ^{13}C-ureumademtest bij 20 asymptomatische patiënten met histologisch bewezen atrofische corpus gastritis werden vergeleken met de resultaten van de *H. pylori*-kweek als referentietest. We vergeleken de resultaten ook met serologie.

De totale nauwkeurigheid van de ^{13}C-ureumademtest vergeleken met de kweek bleek 85.0% te zijn en *H. pylori*-serologie met de kweek liet een nauwkeurigheid zien van 75.0%.

Als de ^{13}C-ureumademtest uitgevoerd werd alleen bij de patiënten met posi-

tieve serologie was de betrouwbaarheid zelfs 92%. De conclusie van dit onderzoek is dan ook dat de ^{13}C-ureumademtest een plaats verdient bij de detectie van een actieve *H. pylori*-infectie bij asymptomatische patienten met atrofische corpusgastritis, bij voorkeur in combinatie met serologie.

Commentaar

De ^{13}C-ureumademtest wordt beschouwd als één van de meest nauwkeurige diagnostische testen om actieve H. pylori infectie aan te tonen bij patiënten met niet-atrofische gastritis.

Echter, bij patiënten met atrofische gastritis is het aantonen van H. pylori infectie moeilijk wegens het progressief verdwijnen van de bacterie. Testresultaten worden duidelijk bepaald door de afnemende kolonisatiegraad van H. pylori als gevolg van de ongunstige atrofische omstandigheden. Het enige teken van een doorgemaakte infectie kan een positieve serologische uitslag zijn hetgeen niet discrimineert tussen een actieve of een doorgemaakte infectie.

Opmerkelijk is dat onze eerstelijnsstudie voor het eerst laat zien dat de ^{13}C-ureum ademtest een goede betrouwbaarheid heeft als test om een actieve H. pylori-infectie aan te tonen bij patiënten met asymptomatische atrofische corpus gastritis, dit in tegenstelling tot andere studies met geselecteerde patiënten. Onze verklaring voor dit verschil is de aselecte, asymptomatische eerstelijnspopulatie zonder H. pylori-eradicatie in de voorgeschiedenis en zonder antibiotica-medicatie de afgelopen drie jaar, beide door de huisarts geverifieerd.

Onderzoeksgegevens verkregen van patiënten die naar de tweedelijn zijn verwezen kunnen niet zonder meer gebruikt worden bij patiënten in de eerste lijn.

Het is vermeldenswaardig dat in theorie de resultaten van deze ^{13}C-ureumademteststudie in de huisartspraktijk van toepassing kunnen zijn op patiënten met artificiële hypo- of achhorhydrie die zuurremmende medicatie slikken, dit in tegenstelling tot gegevens uit de literatuur.

Verder onderzoek bij een grotere eerstelijnspopulatie ten aanzien van de H. pylori-detectie bij atrofische gastritis is gewenst om de richtlijnen aan te passen bij de vaststelling van H. pylori -infectie bij dergelijke patiënten.

In ***Hoofdstuk 7*** wordt ingegaan op de resultaten van een nieuwe test waarbij protonpompremmer (PPI) gestimuleerd pepsinogeen wordt gemeten bij 25 patiënten met serologische atrofische corpusgastritis. De PPI-gestimuleerde pepsinogeenrespons wordt vergeleken met de histologische evaluatie van de corpusbiopten van dezelfde patiënt. De huidige methoden om de ernst van atrofische gastritis aan te tonen worden gefrustreerd door diverse factoren. Gastroscopie met biopsie heeft het bezwaar van steekproef-onvolkomenheid, basaal pepsinogeen het nadeel van variabiliteit en pentagastrine-zuurstimulering het probleem van de patiënt-onvriendelijkheid. Het doel van deze exploratieve studie was om na te gaan of een verminderde serumpepsinogeen-stijging tijdens kortdurend stimuleren met een PPI,

wijzend op verminderde hoofdcelfunctie, gebruikt kan worden om de ernst van corpusatrofie en mogelijk het toegenomen risico op maagkanker vast te stellen.

De serumpepsinogeen A stijging tijdens PPI stimulering blijkt abnormaal laag te zijn bij patiënten met atrofie van het corpus van de maag. Ons onderzoek toont duidelijk aan dat de PPI-gestimuleerde stijging van pepsinogeen A bij patiënten met atrofische corpusgastritis omgekeerd gerelateerd is aan de ernst van de atrofie, met andere woorden, hoe ernstiger de atrofie, hoe geringer de stijging van pepsinogeen A. De hier beschreven eigenschappen samen met de hoge nauwkeurigheid maken de PPI-atrofie test een effectief instrument om de ernst van atrofische corpusgastritis vast te stellen en patiënten te identificeren met corpusatrofie die grenswaarden vertonen van basaal-pepsinogeen.

Commentaar

Het principe van stimulering van functie is een bekend diagnostisch hulpmiddel bij klinisch onderzoek en in de medische wetenschap. Het openbaart zeer nauwkeurig verlies aan functionele cellulaire productiecapaciteit.

Deze studie bevestigt de hypothese dat een verminderde stijging van pepsinogeen A tijdens kortdurend stimuleren met een PPI een marker is voor de ernst van corpusatrofie van de maag. PPI-gestimuleerd pepsinogeen zou een vervanger kunnen zijn voor basaal pepsinogeen als "gouden standaard" voor serologische corpusatrofie. Een verder doelmatigheidsonderzoek moet uitwijzen of de PPI-atrofie test een plaats verdient als complementaire test bij het graderen van de ernst van corpusatrofie van de maag als aandoening met een verhoogd risico.

Het pilotonderzoek in ***Hoofdstuk 8*** beschrijft de vraag of *H. pylori*-infectie betrokken zou kunnen zijn bij het ontstaan van de vroege stadia van ernstige corpusatrofie van de maag die karakteristiek is voor manifeste pernicieuze anemie en die doorgaans *H. pylori*-negatief is.

Wij benaderden deze vraag door de pre-atrofische en pre-pernicieuze fase van auto-immuun gastritis te onderzoeken. Deze voorstadia van auto-immuun gastritis kunnen gevonden worden bij eerste en tweede graads familieleden van patiënten met pernicieuze anemie.

Wij bestudeerden de seroprevalentie van *H. pylori*-infectie en van atrofische corpusgastritis bij 50 eerste en tweede graads familieleden van 8 pernicieuze anaemie-patienten in onze huisartspraktijk en vergeleken dezelfde parameters met die van 45 eerste en tweede graads familieleden van 8 hypertensie-patiënten. Hypertensie heeft als regel geen causale relatie met *H. pylori*-infectie.

Wij vonden géén significant verschil in *H. pylori*-prevalentie tussen de familieleden van patiënten met pernicieuze anemie en de familieleden van de patiënten met hypertensie als controle groep, hetgeen suggereert dat *H. pylori* niet betrokken is bij het ontstaan van het voorstadium van pernicieuze anemie dat later ontaardt in ernstige corpusatrofie.

Het wachten is nog steeds op uitsluitsel over genetische invloeden bij het ontstaan van auto-immuun of *H. pylori*-gerelateerde gastritis.

Commentaar

De kennis over de ware oorzaak en aard van pernicieuze anemie toont nog steeds lacunes waarvan er twee vermeldenswaardig zijn:

Ten eerste, de twijfelachtige rol van H. pylori infectie bij het ontstaan en in stand houden van auto-immuun gastritis.

Onze eerstelijnspilotstudie laat geen significante betrokkenheid zien van H. pylori maar een belangrijke beperking van de studie is het statistisch kleine aantal patiënten. Volgens epidemiologische gegevens kan H. pylori aanwezig zijn in de beginstadia van auto-immuun gastritis. Een lange termijn vervolgstudie inzake autoimmuun gastritis zou wenselijk zijn.

Ten tweede, de onbekende genen, verantwoordelijk voor de aangeboren predispositie voor humane gastritis.

Pernicieuze anemie lijkt een ideale aandoening voor groot-schalig familie-onderzoek, en de gegevens die hieruit voortvloeien zouden toegepast kunnen worden bij auto-immuniteit in het algemeen. De rol hierin van eerstelijnsonderzoek kan alleen erg bescheiden zijn.

Afsluitende opmerkingen

Er is vandaag de dag geen overtuigend bewijs voorhanden dat vroegopsporing van maagatrofie, als precancereuze aandoening, leidt tot reductie van mortaliteit aan de ziekte en verbetering van de kwaliteit van leven. De in dit proefschrift beschreven resultaten kunnen het inzicht in de pathogenese van chronische atrofische gastritis als een multifactorieel proces vergroten. Genetische factoren bij de gastheer bepalen de basale immuunreactie tegen *H. pylori*. Bovendien kunnen milieu factoren deze reactie moduleren en de klinische uitkomst bepalen. Het effect van de behandeling voor *H.pylori* ter preventie van de ontwikkeling van maagkanker bij chronisch geïnfecteerde personen is nog steeds onbekend. Voortdurende research naar dit belangrijke gezondheidsvraagstuk is te rechtvaardigen.

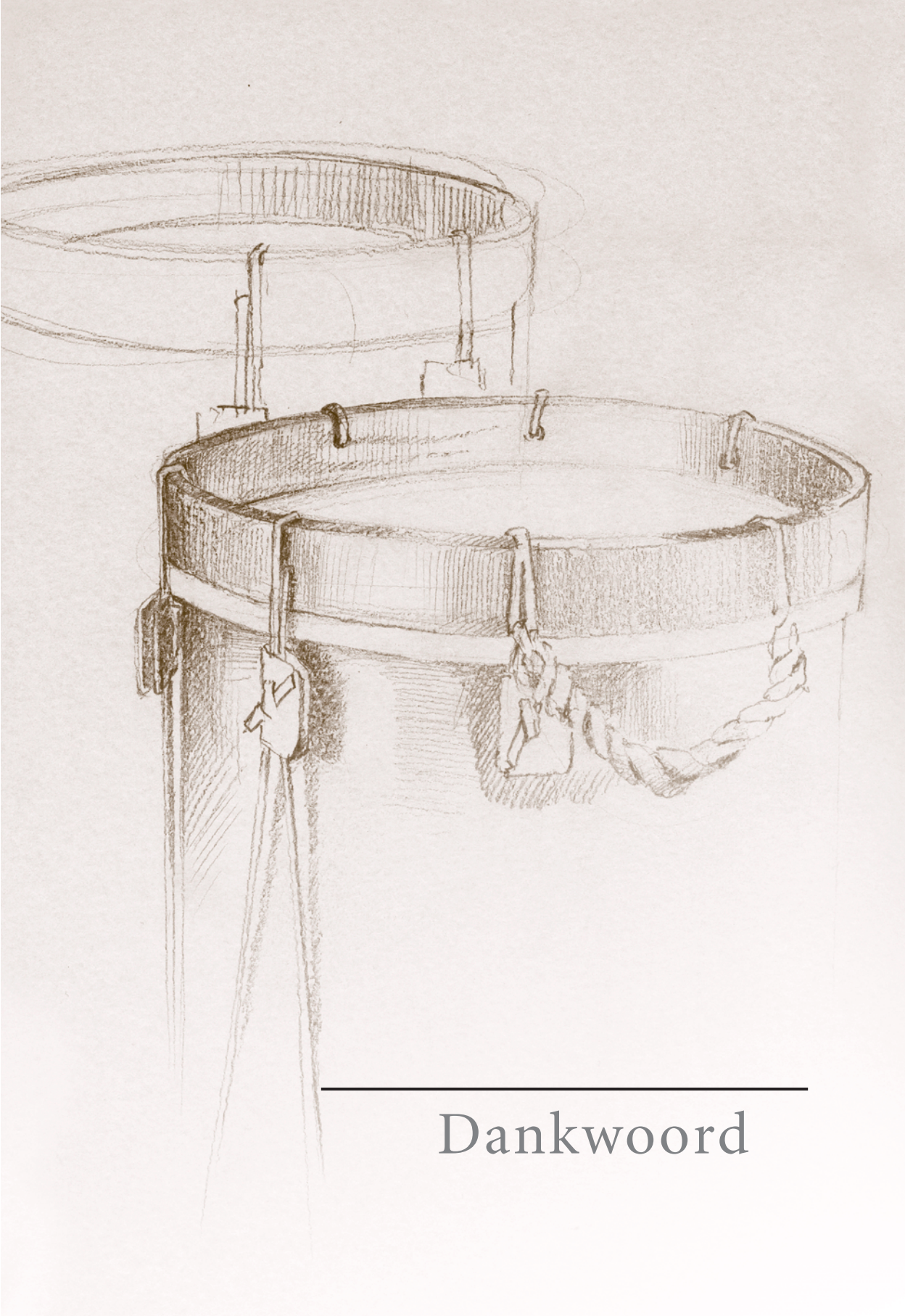
De studies die in dit proefschrift beschreven worden, zijn niet onderworpen aan een bepaling vooraf van noodzakelijke steekproefgrootte en voldoende onderscheidingvermogen. Derhalve is het te vroeg om conclusies te trekken ten aanzien van het algemene nut van het serologische maagonderzoek voor de huisartspraktijk.

De exploratieve studies zijn volledig opgezet en verricht in de eigen praktijk. De gunstige positie van de huisarts in het huidige Nederlandse gezondheidsbestel verdient vermelding. Hij is met recht de aangewezen persoon is om zijn patiënten te motiveren om deel te nemen aan wetenschappelijk onderzoek in de praktijk. De resultaten van dit proefschrift suggereren dat voldoende bewijs is aangedragen om

het gebruik van serologische maagonderzoek in de huisartspraktijk te bepleiten. Het onderzoek is bijzonder verhelderend voor een beter begrip voor de complexe pathofysiologie van de maag. Tevens verschaft het proefschrift inzicht in een meer onderbouwd diagnostisch beleid ten aanzien van maagaandoeningen.

Er bestaat toenemende onderkenning van de noodzaak voor solide research in de eerstelijnsgezondheidszorg met aandacht voor gerandomiseerde en gecontroleerde trials. Voldoende werving van professionals en patiënten voor onderzoek is belangrijk omdat de resultaten dan waarschijnlijk meer generaliseerbaar zijn.

Huidige of ambitieuze aankomende onderzoekers zouden de resultaten van dit proefschrift moeten bevestigen en bekrachtigen door gebruik te maken van een strak georganiseerd onderzoeksprotocol. Dit zal niet alleen de wetenschappelijke honger stillen maar geeft de huisarts in de naaste toekomst ook de gelegenheid de juiste diagnostische methodiek te kiezen in de eerstelijns gastroenterologie.



Dankwoord

◆

It's no misfortune to be born in a duck's nest
from a swann's egg.

(Hans Christian Andersen)

◆

Dankwoord

Een dankwoord schrijven geeft altijd een beetje een ambigue gevoel, opluchting omdat het werk af is en blij om te kunnen bedanken maar ook een gevoel van jammer dat alles achter de rug is, dat je de gezonde drive en spanning straks mist die een promotie met zich meebrengt en dat je een andere intellectuele uitdaging moet zoeken voor alle vrijkomende energie en tijd.

Eindelijk ligt het er dan, mijn proefschrift. Een proefschrift maak je zeker niet alleen. Ik ben dan ook allen, die op enigerlei wijze een steentje hebben bijgedragen aan de totstandkoming ervan, zeer erkentelijk.

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Mia de Koeijer die me enthousiast heeft geholpen met het afnemen van alle bloedmonsters in de huisartspraktijk;

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Verder, natuurlijk dank aan mijn beide associées Dieneke van der Tempel en Willem-Jan Kronenberg voor de mentale en fysieke steun om mijn promotie tot een goed einde te brengen. Jullie gaven mij de vrijheid en mogelijkheid om mijn onderzoek te verrichten terwijl de praktijk moest doordraaien. Jullie bereidheid om paranimf en ceremoniemeester te zijn tijdens de promotie en de daarbij behorende festiviteiten stemt mij tot grote vreugde.

Ook mijn praktijkassistentes, Riet, Corrie, Rianne, Bernarda, Lucinda en Marian wil ik danken voor hun medeleven al de jaren dat mijn onderzoek duurde. Jullie vroegen vaak "Hoe lang duurt het nu nog"? Eindelijk kan ik zeggen "Niet lang meer".

De steun die je als promovendus ondervindt van je partner lijkt vanzelfsprekend, maar is dit natuurlijk niet altijd. Het vele jaren werken aan mijn promotieonderzoek en het schrijven van mijn proefschrift was slechts mogelijk bij de gratie van veel uithoudings- en incasseringsvermogen van mijn echtgenote voor wier liefdevolle steun ik haar ten zeerste dankbaar ben. Het doen van onderzoek en het schrijven van publicaties werd gelukkig ook door haar beschouwd als hobby van mij. Het is inderdaad een fascinerende hobby geworden.

Bij het schrijven van mijn proefschrift heb ik talloze artikelen, tijdschriften en boeken geraadpleegd. Onlangs las ik dat iets uit één boek overschrijven plagiaat is, en dat iets uit verschillende boeken overschrijven, wetenschappelijk werk is. Ik koester hiermee de gedachte dat ik vandaag in mijn dissertatie de wetenschap heb gediend.

A. Korstanje

Curriculum vitae

De auteur van dit proefschrift werd geboren op 19 juni 1947 te Pangkalpinang op het eiland Bangka (Indonesië) alwaar zijn vader beroepsmilitair was bij het Koninklijk Nederlands Indisch Leger. In 1950 repatrieerde de familie naar Nederland. Een plezierige jeugd werd doorgebracht in het Zeeuwse dorp 's-Gravenpolder. In 1965 behaalde hij het diploma Gymnasium β, aan het Goese Lyceum te Goes en in het zelfde jaar begon hij met de studie Geneeskunde aan de Universiteit van Amsterdam. Tijdens zijn studie was hij 2 jaar, van 1967-1969, kandidaats-assistent op de afdeling Anatomie & Embryologie.

In 1971 werkte hij een half jaar als semi-arts-assistent op de afdeling Interne Geneeskunde bij Prof. Borst in het historische Binnen Gasthuis in Amsterdam. Het artsexamen werd afgelegd in april 1973.

De dienstplicht werd daarna vervuld als reserve officier-arts bij de Koninklijke Landmacht.

Na een periode van praktijkwaarneming en een huisarts-assistentschap vestigde hij zich in 1975, associatief, als apotheekhoudende en verloskundig actieve huisarts in zijn ouderlijk dorp 's-Gravenpolder. Inmiddels, na 31 jaar is hij nog steeds full-time praktiserend huisarts, inmiddels geassocieerd met twee jongere collega's.

De altijd gekoesterde wens om te promoveren kreeg vastere vorm in het begin van de jaren 90. De voorzet om met de wetenschap in zee te gaan kwam van Dr. G. den Hartog, toendertijd als gastroenteroloog werkzaam in het Oosterscheldeziekenhuis in Goes. Hij vroeg aandacht voor het epidemiologisch fenomeen van de boven-gemiddelde prevalentie van maagkanker in de Zak van Zuid-Beveland (gegevens IKR), een fenomeen wat feitelijk geëvalueerd moest worden op eerstelijnsniveau. Maagserologisch onderzoek leek hiertoe de geëigende onderzoeksmethodiek. Via bemiddeling van Dr. den Hartog werd welwillende wetenschappelijke ondersteuning geboden door Prof. dr. C.H.W.H. Lamers en Dr.I. Biemond, respectievelijk hoofd en staflid van de afdeling Maag-, Darm- en Leverziekten van het Leids Universitair Medisch Centrum. Onder supervisie van Prof. Lamers werd in 1995 een aanvang gemaakt met het onderzoek waarvan de resultaten in dit proefschrift zijn samengevat.