SUMMARY, CONCLUSIONS AND FUTURE PERSPECTIVES

Gastro-oesophageal reflux disease (GORD) is a very common disorder causing symptoms like heartburn, belching and regurgitation, painful food passage, or even hampered food passage, depending on the severity of the reflux, the sensitivity of the oesophageal mucosa and any possible changes in the anatomy. In the normal population many need acid neutralizing, acid suppressive, or prokinetic drugs, either on prescription or "over the counter". GORD, however is more than heartburn, problems with food passage or oesophagitis. There are extra-oesophageal, in particular respiratory manifestations; and, for some, there is an increased risk to develop cancer. This thesis is about these two aspects of GORD.

A combined action of GOR, neural reflexes and the release of active substances in the upper aero-digestive tract seems to induce or aggravate airway disease, both on ear-nose-throat level and on tracheo-bronchial level. Though the symptoms have been already described a long time ago, hard facts and solid figures have been scarce or contradictory for many years. **Chapter I** gives an overview of the problem of so-called gastric asthma. Symptomatology and clinical outcome are the endpoints in many studies. Pathophysiologically two mechanisms have been studied by several researchers: microaspiration of refluxed gastric contents into the airways, and vagally transmitted reflexes, leading to bronchoconstriction. A third mechanism is less well examined: the increase of airway hyperresponsiveness, possibly due to a heightened vagal tone or the associated release of inflammatory mediators. Intervention studies, both medical and surgical, have not been unequivocal. Nevertheless, there is a group of patients with both asthma and GORD, who may benefit from anti-acidreflux therapy. These are the patients with difficult to treat asthma, adult onset asthma and non-allergic asthma. In clinical practice the aim of therapy is healing of a disease, or if impossible improvement of symptomatology. Symptoms however, are by definition subjective. In addition to symptoms, objective measures are needed. **Chapter II** reports the results of our double-blind randomized prospective study. A group of asthma and COPD patients with severe airway hyperresponsiveness and increased acid gastro-oesophageal reflux was treated with profound acid suppressive medication, omeprazole 40 mg b.i.d. for three months or with placebo. Treatment with omeprazole did not improve airway hyperresponsiveness (as measured by a fall of 20% in FEV_1 after methacholine challenge), or any of the other tested respiratory parameters, like peak expiratory flow (PEF), vital capacity, and reversibility by ipratropium bromide. With regard to symptoms, only heartburn improved significantly whereas respiratory symptoms did not. In conclusion the results do not support a role in general for intensive anti-reflux therapy in asthma or COPD patients with severe airway hyperresponsiveness.

Guinea pig experiments have shown recently that oesophageal stimulation with hydrochloric acid causes a neurogenic inflammation of the airways, that was mediated by the release of active substances, including probably substance P, via a neural pathway involving the vagal nerve [1]. As regards the vagally mediated reflex, asthmatics with

GORD appear to have symptoms of autonomic dysfunction with a heightened vagal tone, which may be a factor in the airway response to acid reflux [2]. This explanation is, however considered improbable by others [3]. A large epidemiological study based on questionnaires showed that GOR symptoms and bronchial hyper-responsiveness (as well as irritable bowel syndrome) occur more often together than expected [4]. The investigators explain this finding not with a causal relation, but with a common, still unknown, causal factor that produces symptoms in more than one system. Candidates could be a dysfunction in smooth muscle, a neuromuscular disorder, or a shared inflammatory aetiology.

So with regard to gastric asthma: which patients benefit most from anti-reflux medication? If the prevalence of GORD is so high in asthmatics, why do so few benefit from GOR-directed therapy? What is needed is a better characterization of the patients, and more insight in the pathophysiology. There must be more than a simple vagal reflex and microaspiration. What is the role of the non-adrenergic non-cholinergic (NANC) innervation? What is the exact role of nitric oxide (NO), synthesized by either constitutive or inducible nitric oxide synthase (c-NOS and i- NOS)? Inflammatory mediators are released, but can we counteract their action or suppress their release? For the time being, there is no need to treat every asthmatic for GORD. But one has to think of it in case of difficult to treat asthma, adult onset asthma, non-allergic asthma, and maybe in case of night time aggravations; this counts when there are no typical GOR symptoms. Obviously the patient needs to be examined for GORD when there are typical symptoms, and to be treated adequately. It may be somewhat disappointing, but one has to be satisfied with symptom improvement only, without any objective improvement in lung function [5].

Chapter III contains an overview of literature on Barrett's oesophagus (BO) and adenocarcinoma of the oesophagus. BO is caused by chronic gastro-oesophageal acid reflux. The role of duodenal reflux is controversial. BO is characterized by columnar metaplasia of the epithelium. Three types of metaplasia can be recognized: the specialized or intestinal type, the gastric-junctional or cardia type, and the gastric-fundic type. More recently the definition of BO has been restricted to columnar metaplasia of the specialized-intestinal type with its pathognomonic goblet cells [6]. The incidence of adenocarcinoma of cardia and oesophagus is increasing. Patients with BO have a 30 to 125-fold increased risk for oesophageal adenocarcinoma compared with the general population. Tumours develop from specialized-intestinal type metaplasia with low-grade dysplasia and high-grade dysplasia as intermediate stages. This histological change is to a certain degree accompanied by genomic instability with aneuploidy and increased G2/tetraploid fractions. Overexpression of the p53 protein, due to a mutation of the p53 gene is often seen in high-grade dysplasia and cancer. In several studies increased epithelial cell proliferation was found in dysplasia and adenocarcinoma. Moreover in a few studies cell proliferation was higher in intestinal type than in gastric type metaplasia. More or less parallel to the proliferation rate, an increase in the expression of the transforming growth factor alpha (TGF-α) , and of the epidermal growth factor receptor (EGF-r) was observed.

Oesophagitis and BO are caused by reflux of gastric contents into the oesophagus, of which acid is the most harmful component. Chemotherapy can induce mucositis of the GI-tract, especially of the mouth and of the oesophagus. Some investigators observed the development of BO in patients treated with chemotherapy for breast cancer. In **Chapter IV** the results of our study on this subject are presented. Our study population consisted of two groups: one group with testicular carcinoma for which they were treated with highly emetogenic chemotherapy; and another with breast cancer treated with high dose chemotherapy with subsequent mucositis, in preparation for autologous bone marrow transplantation. Fortunately we found no increased prevalence of BO in both groups. Others came at about the same time as we to the same conclusion, checking on adjuvant chemotherapy for breast cancer [7]. Thus screening for BO after chemotherapy is not generally indicated.

In normal healthy tissue there is a delicate balance between cell proliferation and programmed cell death (apoptosis). Both phenomena are under the influence of many regulatory mechanisms, with among other things autocrine, paracrine and genetic aspects. In premalignant and malignant tissue these mechanisms are disturbed, leading to increased cell proliferation and altered apoptosis. In **Chapter V** we report our methodology of measuring epithelial cell proliferative activity in BO, and its correlation with accepted cancer risk markers. Incorporation *in vitro* of 5-bromodeoxyuridine (BrdU) into the nucleus is comparable and equivalent to the incorporation of tritiated thymidine; both are incorporated into the DNA during the S-phase of the cell cycle. The advantage of the BrdU technique is that no radioactive materials are used, and that the label is in the tissue itself, with the possibility to relate labeling directly with morphology. The endoscopically taken biopsies were embedded in glycol methacrylate (Technovit 8100). Slides of 2 µm thick were used for immunohistochemical detection of BrdU, for hematoxylin-eosin (H&E) and for periodic acid-Schiff (PAS) staining. In each sample 4000 nucleated luminal epithelial cells and 4000 nucleated crypt cells were counted. Crypt and surface epithelium labeling index (LI) were determined separately in biopsies taken from different levels of BO. The length of BO correlated positively with the severity of erect acid reflux. Inter- and intra-observer variation in LI determination were within acceptable limits.We found the LI increasing from gastric-fundic to gastric-junctional to intestinal-specialized metaplasia. The difference was statistically significant for crypt epithelium. We found a higher LI at the surface in case of dysplasia compared to no dysplasia, and in the more distal part of BO. Crypt LI was correlated with the length of BO, and with the type of BO metaplasia. So the LI correlated with well-known risk factors for Barrett's carcinoma. This method of measuring proliferation appears to be a reliable tool and hence may be a valid intermediate marker for intervention studies.

There is a positive correlation between the length of BO and the risk for adeno-carcinoma. There is also a correlation between length of BO and the severity of acid reflux. According to this line of reasoning control of acid reflux might induce regression of BO, and decrease cancer risk. In **Chapter VI, VII and VIII** we report our data of an intervention study. We performed a randomized double-blind prospective study in which patients with BO and acid reflux were treated in parallel groups for two years with either omeprazole 40 mg b.i.d. or with ranitidine 150 mg b.i.d.. Treatment with high dose

omeprazole eliminated acid reflux almost completely, whereas treatment with ranitidine in this conventional dose resulted in an acceptable symptom reduction with a still pathologically increased acid reflux percentage. Endoscopic regression was assessed in length as well as in area of BO. Length is relatively easy to measure, relating the distances of all "landmarks" to the incisors. The squamo-columnar junction can however be rather irregular in BO, with islands of squamous epithelium in BO, and islands of metaplasia in squamous epithelium. Where others counted islands, we tackled this problem by making drawings of BO during endoscopy, from which we calculated the area of BO, as described in **chapter VI**. We found a small but steady decrease in BO during treatment with omeprazole: 8% in two years which was statistically significant. In the ranitidine patients no change in length or area of BO was observed. Indeed the observed decrease is small, and we do not know whether this improvement will continue beyond the two study years with continuation of medication. But since regression occurred gradually during the two years, a further reduction of BO may be expected. A combination of some form of mucosa ablation (for instance by means of heater probe, laser, or argon plasma coagulation) with strong acid suppression may lead to better results, as it is reported by others. Unfortunately, despite the promising results, it was found that in 30% columnar metaplasia was still present hidden under the overlying squamous epithelium [8-11].

Cancer risk reduction might be achieved by macroscopic regression of BO, it may also be achieved by changes for the better in BO itself, represented by a reduction of cell proliferative activity. In the same two groups of patients in this double blind prospective study we measured the cell proliferative activity in BO, using *in vitro* labeling with BrdU. The study period was 24 months, during which the patients were treated with either omeprazole 40 mg b.i.d. or ranitidine 150 mg b.i.d. Cell proliferation was measured in biopsies, taken at 0, 3, 9, and 24 months. We assessed the LI in the lowest (*i.e.* 3 cm above the gastro-oesophageal junction) and the highest (just below the Z-line) taken biopsy of BO, for the luminal part and the crypt part separately. In **chapter VII** we report the results. In the lower biopsy we saw a significant increase in luminal LI in case of persistent acid reflux, whereas in case of eliminated acid reflux no significant change was observed. There was an increase of crypt LI in both groups, statistically not different from each other. In the proximally taken biopsies luminal LI increased somewhat during persistent reflux, but not significantly so; again in the group without reflux it remained stable. Crypt LI however increased significantly in the omeprazole treated group. Elimination of acid reflux for two years thus had a beneficial effect on cell proliferation with less proliferation in the luminal part and more of the proliferation confined to the crypts of Barrett's epithelium. This prevention of a further increase of proliferative activity at the luminal side of BO may indicate a reduction of cancer risk.

In a large percentage of BO with high-grade dysplasia and in adenocarcinoma of the oesophagus immunohistochemical overexpression of p53-protein is found, whereas this is rarely found in non-dysplastic BO. During oncogenesis genomic instability arises. Mutation of the tumor suppressor gene p53 can lead to dysfunction of the gene and to immunohistochemical overexpression of p53-protein. By slowing down proliferative activity the occurrence of mutations, particularly of the p53 gene might be averted. Of the, possibly many, growth factors involved, we have studied the expression of the transforming

growth factor-α (TGF-α) and of the epidermal growth factor receptor (EGF-r), again in patients who were treated for two years with either high dose omeprazole or conventional dose ranitidine, so one group with acid reflux eliminated and another with persistent acid reflux. In **Chapter VIII** we present our results. In none of the 109 baseline biopsies overexpression of p53-protein was observed. After 24 months, again in the omeprazole treated as well as in the ranitidine treated group no overexpression was found. No high-grade dysplasia was present in the baseline biopsies since this was an exclusion criterion; at 24 months no high-grade dysplasia was found in any of the biopsies either. With these results we cannot tell whether acid reflux elimination can prevent mutations in the p53 gene. Determination of TGF-α and EGF-r expression was done only in the 24 month biopsies. TGF-α expression was significantly stronger at the surface than in the crypt; in contrast with other authors we found a stronger expression in gastric type metaplasia tghan in intestinal type. This might be due to the acid suppressive therapy, putting a check on cell proliferation in intestinal metaplasia in particular. There was however no significant difference between the two treatment groups. EGF-r expression showed even less variation. EGF-r expression in gastric type metaplasia was weaker in the omeprazole-treated group than in the ranitidine group. Overall we did not find a significant influence of acid reflux elimination on the expression of TGF-α and EGF-r. Either there is no such influence, or the observed expression is the result of a number of mechanisms with opposite effects.

A new aspect in the discussion on pathogenesis of BO is the role of the *Helicobacter pylori* bacterium, especially the *cagA*-positive strain. Recent epidemiological studies favour a protective role of *H.pylori* against severe GORD, BO and adenocarcinoma of the oesophagus and the cardia, whereas *H.pylori* is regarded to be a malignancy promoting factor for gastric cancer of the diffuse and the intestinal type [12] and for MALToma of the stomach. The role of *H.pylori* in uncomplicated GORD is still not clear, despite some provocative articles, reporting that eradication of *H.pylori* would increase the incidence of oesophagitis. However, other investigators could not confirm these observations. Moreover eradication would render the pH-increasing effect intra-gastrically of acid suppressive medication less effective. We investigated whether there was a difference between *H.pylori*-negative and *H.pylori*-positive patients regarding symptoms of GOR and percentage of acid reflux per 24 hours, and whether there was a difference between the two during treatment with omeprazole or ranitidine. In **Chapter IX** we present the results. Patients, who were *H.pylori*-positive or *H.pylori*-negative based on a serum IgG ELISA, were treated with omeprazole 40 mg b.i.d. or with ranitidine 150 mg b.i.d.. They were interviewed for their GOR symptoms and underwent 24 hour pH-metry at two occasions (at baseline and during therapy). At baseline oesophageal acid exposure and GOR symptoms did not differ between the *H.pylori*-negative and the *H.pylori*-positive patients. Treatment with the two acid suppressive drugs resulted as expected in almost elimination of acid reflux in the omeprazole group, and a smaller reduction in acid reflux in the ranitidine group. There was however no significant difference in either treatment group between *H.pylori*-negative and *H.pylori*-positive patients. As for the course of symptoms during therapy, no significant difference was observed between the two groups. It is therefore not necessary to modify the medication dosage because of the *H.pylori* status in the treatment of patients with GORD.

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With respect to Barrett's oesophagus many questions have not been answered yet. Why do some develop columnar metaplasia in the oesophagus as a consequence of gastro-oesophageal reflux? As a group these patients have more acid reflux, according to some more duodenal reflux, more often a hiatal hernia, and more often impaired peristalsis and lower oesophageal sphincter function. The quantity of acid reflux is undoubtedly an important factor. This is confirmed by the observation that symptomatic GORD itself is a strong risk factor for oesophageal adenocarcinoma [13]. But more factors must be involved in the pathogenesis because of the comparable severity of reflux in patients with severe oesophagitis but without Barrett's metaplasia [14]. What is the role of *Helicobacter pylori*, especially of the *cagA*-positive strain [15]? Its presence seems to protect against oesophagitis and adenocarcinoma of the oesophagus [16-19]. Two recent reports, however, could not confirm this protective effect of *H.pylori* against reflux disease [20,21]. Maybe there is also a familial constitutional factor [22,23]. The group at risk for adenocarcinoma is better defined now, since we know that only specialized-intestinal metaplasia is the culprit. Can we accentuate screening and surveillance? Is surveillance sensible or useless? Opinions on this matter differ. Most of the oesophageal and cardia adenocarcinomas are diagnosed when they are symptomatic and in an advanced stage. Only a small minority of known BO patients dies of adenocarcinoma [24]; on the other hand surveillance results in earlier detection and hence a more favourable tumor stage at the time of diagnosis and better chances for survival [25,26]. Do we have to remove the metaplastic epithelium? Mucosal ablation by any of the used methods (laser coagulation, argon beamer coagulation, heater probe, photo-dynamic therapy) in combination with proton pump inhibitors for acid suppression looks promising. This would also mean that surveillance could be justified for those who are regarded unfit for surgical treatment but can stand endoscopic therapy. But this treatment modality has complications, and in addition to this in about 30% remnants of intestinal metaplasia were found hidden under squamous epithelium [8,9,11]. We need a longer follow-up to recommend this as a standard procedure. A large prospective trial would be preferable, comparing a) acid reflux elimination; b) mucosal ablation with reflux elimination; and c) adequate control of reflux oesophagitis, with high-grade dysplasia and carcinoma as endpoints.

A practical guideline for present-day patient care may be to perform upper gastro-intestinal endoscopy at least once in case of longstanding (which means more than 5 years) GORD, or if the patient is older than 50 years; if there is Barrett's metaplasia of the specialised-intestinal type without dysplasia to repeat endoscopy after one year when newly diagnosed, than every 2 to 3 years; if there is low-grade dysplasia as a new finding to repeat it after 6 months and, when stable, then every year; if there is high-grade dysplasia as a new finding to repeat endoscopy after 3 months for confirmation. If high-grade dysplasia is still present, surgical resection should be considered seriously; and if resection is not feasible endoscopic mucosal ablation is an option, for the time being preferably within a study protocol. Obviously acid suppressive therapy must be given to those with GORD symptoms. There are good reasons to treat patients without clear symptoms too: the patient may not have recognized his symptoms sufficiently as GOR-related. And elimination of acid gastro-oesophageal reflux appears to be favourable for metaplastic Barrett's epithelium.