Nocturnal Gastroesophageal Reflux: Respiratory Symptoms and Obstructive Sleep Apnea

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Thesis for the degree of Philosophiae Doctor

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Tengsl vélindabakflæðis að nóttu við öndunarfæraeinkenni og kæfisvefn

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With a burning sensation

Ágrip

Inngangur: Vélindabakflæði að nóttu (VBn) tengist bæði öndunarfæra–einkennum og einnig einkennum svefnháðra öndunartruflana (SHÖ), en ekki er vitað hvort um orsakatengsl sé að ræða. Markmið verkefnisins var að skoða tengsl VBn við öndunarfæraeinkenni og einkenni SHÖ, sem og breytingar á blástursprófi, svefnrannsókn og lífmerki frá öndunarfærunum.

Aðferðir: Þátttakendur í tveimur stórum alþjóða rannsóknum sem byggja á slembiþýði voru rannsakaðir: „Burden of obstructive lung disease (BOLD)“ og „European community respiratory health study (ECRHS)“ fyrsti (I) og annar (II) hluti. Að auki var einstaklingum með VBn í þriðja (III) hluta ECRHS á Íslandi boðið í umfangsmiklar viðbótar rannsóknir, auk svipað margra paraðra viðmiða. Í öllum rannsóknum voru þátttakendur taldir hafa VBn ef þeir greindu frá einkennum brjóstsviða eða nábíts að nóttu. Öndunarfæraeinkenni og einkenni SHÖ voru metin með spurningalistum, blóðsýnum safnað og blástursþróf framkvæmd. Þátttakendur í ECRHS III VBn undirþýði gengust að auki undir svefnrannsókn, og gáfu tvenns lags sýni af útöndunarlofti: Vökvaþéttisýni og smáagnasýni. Hluti þessa þýðis gekkst einnig undir 24 tíma samviðnáms- og pH vélindamælingu.

Niðurstöður: Einkenni astma og berkjubólgu voru algengari meðal þátttakenda með VBn en viðmiðunarhóps, sem og versnanir öndunarfæraeinkenna. Einkenni SHÖ voru einnig algengari meðal þátttakenda með VBn. Á níu ára tímabili þróuðu þátttakendur með VBn um það bil tvöfalt oftar með sér öndunarfæraeinkenni og einkenni SHÖ en viðmiðunarhópur án VBn. Engar skýrar breytingar fundust á blástursprófi. Hlutlægt mældar hrotur voru algengari meðal þátttakenda með VBn. Í vökvaþéttisýnum voru pepsín, efni P (substance P) og 8-ísóprostan (8-isoprostane) í meira magni meðal þátttakenda með VBn. Þátttakendur með bæði VBn og næturhósta voru með aukið efni P í vökvaþéttisýnum. Albúmín og lungnablöðruseytisprótein A voru í minna magni í smáagnasýnum þátttakenda með VBn. Þessar niðurstöður voru óháðar líkamsþyngdarstuðli.

Ályktun: Vélindabakflæði að nóttu tengist öndunarfæraeinkennum og einkennum öndunarerfiðleika í svefni. Viðvarandi vélindabakflæði að nóttu er áhættuþáttur fyrir að öndunarfæraeinkenni og einkenni öndunarerfiðleika í svefni komi fram. Vélindabakflæði að nóttu tengist einnig marktækt hrotum samkvæmt svefnmælingu, sem bendir til aukins öndunarerfiðis í svefni. Lífefnamælingar á vökvaþéttisýnum og smáagnasýnum útöndunarlofts gefa vísbendingar um að bólga í loftvegum sé ein skýring þessara tengsla.

Lykilorð:

Vélindabakflæði að nóttu, Kæfisvefn, Astmi, Bólga í loftvegum, Faraldsfræði

Abstract

Introduction: Nocturnal gastroesophageal reflux (nGER) is associated with respiratory symptoms and sleep-disordered breathing (SDB), but a causal relationship has not been established. The aim of this project was to investigate the association between nGER and respiratory and SDB symptoms, as well as changes in lung function, sleep study and respiratory biomarkers.

Methods: Participants in two European general population cohort studies were studied: The Burden of Obstructive Lung Disease (BOLD), and the European Community Respiratory Health Survey (ECRHS) parts one (I) and two (II). Additionally, participants with nGER in part three (III) of the ECRHS in Iceland were invited for further studies, and similarly as many paired controls. Subjects were identified as having nGER if they reported nocturnal heartburn or regurgitation. Respiratory and SDB symptoms were assessed by questionnaires, blood samples drawn and spirometries performed. Only the ECRHS III nGER subcohort underwent a home sleep study, and collected exhaled breath condensate (EBC) and particles in exhaled air (PEx) samples. A subgroup underwent a 24 hour esophageal impedance-pH measurement.

Results: Asthma and bronchitis symptoms were more common among nGER subjects than controls, as were exacerbations of respiratory symptoms. SDB symptoms were more common among nGER subjects. Under a nine year follow-up, subjects with persistent nGER developed respiratory and SDB symptoms roughly twice as often as those without nGER. No consistent differences were found in lung function tests. Objectively measured snoring was more common among subjects with nGER. Pepsin, substance P and 8-isoprostane in EBC were higher among nGER subjects. Subjects with both nGER and nocturnal cough had increased substance P in EBC. Albumin and surfactant protein A in PEx were lower among nGER subjects. These findings were independent of BMI.

Conclusion: In the general population, nGER is associated with respiratory and SDB symptoms. Having persistent nGER increases the risk of developing respiratory and SDB symptoms. Also, nGER is associated with more measured snoring, indicating increased respiratory effort during sleep. Biomarker measurements in EBC, PEx and serum indicate that airway inflammation is a plausible underlying cause.

Keywords:

Nocturnal gastroesophageal reflux, Obstructive sleep apnea, Asthma, Airway inflammation, Epidemiology

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The path towards a PhD is long and requires the support of a remarkably large group of people to succeed. Even the hundreds of participants in our research projects play a central role and have my gratitude. Here I will try to thank all those who helped me on this long and winding road.

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Special thanks go to other delegates of my PhD committee, Bryndís Benediktsdóttir, Sigurður Júlíusson and Einar Stefán Björnsson. Their support, ideas and commitment have been vital to my work and helped me develop as an independent researcher. I also want to thank especially Ísleifur Ólafsson and Elizabeth Cook for their enormous assistance in all aspects of our biomarker studies. Ísleifur was also my first mentor in the world of research, as he mentored me during my first research project in 2008, a project on the dimerization of cystatin C, which likely ignited the spark for my future research. On that project I also received guidance from Anders Grubb, then professor at Lund University, and my brother-in-law Gustav Östner, who was my main instructor for that project. Thank you all for that great introduction!

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Contents

List of abbreviations

GER	Gastroesophageal reflux
GERD	Gastroesophageal reflux disease
nGER	Nocturnal gastroesophageal reflux
PPI	Proton pump inhibitor
LPR	Laryngopharyngeal reflux
RFS	Reflux finding score
24h MII-pH	24 hours esophageal multichannel pH-impedance
COPD	Chronic obstructive pulmonary disease
FEV1	Forced expiratory volume in one second
FVC	Forced vital capacity
BHR	Bronchial hyperresponsiveness
DLCO	Diffusion capacity of carbon monoxide
BAL	Bronchoalveolar lavage
OSA	Obstructive sleep apnea
AHI	Apnea-hypopnea index
PAP	Positive airway pressure
CPAP	Continuous positive airway pressure
LLMI	Lipid laden macrophage index
PEx	Particles in exhaled air
PExA	Particles in exhaled air method
SDB	Sleep-disordered breathing
BOLD	Burden of obstructive lung diseases initiative
ECRHS	European community respiratory health survey
RDQ	Reflux disease questionnaire
EBC	Exhaled breath condensate
FeNO	Nitric oxide in exhaled air
N-GSSIQ	Nocturnal gastroesophageal reflux disease symptom severity and impact questionnaire

ESS	Epworth sleepiness scale
SAGIC	Sleep apnea global initiative consortium
NHANES	United States national health and nutrition examination survey
LLN	Lower limit of normality
CRP	C-reactive protein
IL-8	Interleukin 8
ELISA	Enzyme-linked immunosorbent assay
SP-A	Surfactant protein A
PBS	Phosphate-buffered saline
BSA	Bovine serum albumin
BMI	Body mass index
ICC	Intraclass correlation
SD	Standard deviation
Coef	Coefficient
IQR	Interquartile range

List of figures

List of tables

List of original papers

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I-V):

I. OI Emilsson, C Janson, B Benediktsdottir, S Juliusson, T Gislason. “Nocturnal gastroesophageal reflux, lung function and symptoms of obstructive sleep apnea: Results from an epidemiological survey”. *Respiratory Medicine* 2012; 106 (3): 459-466.

II. OI Emilsson, A Bengtsson, KA Franklin, K Toren, B Benediktsdottir, A Farkhooy, J Weyler, S Dom, W De Backer, T Gislason, C Janson. “Nocturnal gastro-oesophageal reflux, asthma and symptoms of OSA: a longitudinal, general population study”. *European Respiratory Journal* 2013; 41 (6): 1347-1354.

III. OI Emilsson, B Benediktsdottir, I Olafsson, E Cook, S Juliusson, S Berg, L Nordang, ES Bjornsson, S Gudlaugsdottir, AS Gudmundsdottir, C Janson, T Gislason. “Definition of nocturnal gastroesophageal reflux for studies on respiratory diseases”. *Scandinavian Journal of Gastroenterology* 2016; *51* (5): 524-530.

IV. OI Emilsson, B Benediktsdóttir, I Olafsson, E Cook, S Juliusson, ES Bjornsson, S Gudlaugsdottir, AS Gudmundsdottir, E Mirgorodskaya, E Ljungstrom, ES Arnardottir, T Gislason, C Janson, AC Olin. „Respiratory symptoms, sleep-disordered breathing and biomarkers in nocturnal gastroesophageal reflux”. *Respiratory Research* 2016; 17:115.

Additional review paper in thesis:

V. OI Emilsson, T Gislason, AC Olin, C Janson, I Olafsson. “Biomarkers for gastroesophageal reflux in respiratory diseases”. *Gastroenterology Research and Practice* 2013; 2013: ID (148086).

Declaration of contribution

Paper I: I participated in planning this analysis on existing research data, chiefly with Þórarinn Gíslason, Christer Janson and Bryndís Benediktsdóttir. I performed the statistical analysis together with Christer Janson. I drafted the paper and participated in all revisions of the paper with the co-authors.

Paper II: I participated in planning this analysis on existing research data, chiefly with Þórarinn Gíslason, Christer Janson and Bryndís Benediktsdóttir. I applied for a widened ethics approval. I applied for partial funding. I performed the statistical analysis. I drafted the paper together with Anna Bengtsson and participated in all revisions of the paper with the co-authors.

Paper III: I planned this study together with my PhD committee. I applied for the ethics approval. I applied for the funding. Together with the research assistants, Hjördís Sigrún Pálsdóttir and Helga Hjartardóttir, I met the participants and collected the data, but did not perform the biomarker analyses. I performed the laryngoscopies, which were later scored by Sigurður Júlíusson, Sören Berg and Leif Nordang. I performed the statistical analysis. I drafted the paper and participated in all revisions of the paper with the co-authors.

Paper IV: I planned this study together with my PhD committee. I applied for the ethics approval. I applied for the funding. Together with the research assistants, Hjördís Sigrún Pálsdóttir and Helga Hjartardóttir, I met the participants and collected the data, but did not perform the biomarker analyses. I performed the statistical analysis. I drafted the paper and participated in all revisions of the paper with the co-authors.

Paper V: I designed the format of this review and perspective with Ísleifur Ólafsson. I collected and read all relevant references and drafted the paper and participated in all revisions of the paper from the co-authors.

1 Introduction

1.1 What is gastroesophageal reflux?

Ever since the first century A.D. heartburn has been recognized in the literature, when the Roman scientist Pliny the Elder recommended coral powder as a treatment for heartburn (Richter & Castell, 2012). Both heartburn and regurgitation have been described in the medical literature for centuries, often with vivid descriptions. The following is the description of a patient with gastroesophageal reflux disease from 1933 (Rayner, 1933):

> Mrs. M., two-para, seven months pregnant, complained of very severe heartburn, aggravated by leaning forward or by greasy food. The heartburn was very much worse in bed at night, and kept her awake. She vomited every night.

The earliest documentation the authors found of regurgitation and heartburn was from 1843, where the two were considered as distinct phenomena. Heartburn was considered a symptom of excessive acid in the stomach and said to be very common. Excessive regurgitation, on the other hand, was regarded primarily as a variety of hysteria, and the persons affected were even to be classified as ruminating animals ([Author not listed], 1843). Shortly after 1930, heartburn began to evoke special interest, predominantly as a pregnancy-related condition (Rayner, 1933). However, it was not until 1941 that reflux of gastric contents into the esophagus was hypothesized as a cause, which is today known to be the main factor causing heartburn (Vakil et al., 2006; Williams, 1941). More specifically, we now know these episodes are caused by intermittent, transient relaxations in the lower esophageal sphincter, allowing gastric contents to reflux into the esophagus (Moayyedi & Talley, 2006) (Figure 1). Today, gastroesophageal reflux (GER) disease (GERD) is defined as the backward flow of gastric contents into the esophagus, causing causing troublesome symptoms and/or complications. The cardinal symptoms are heartburn and regurgitation of gastric contents (Vakil et al., 2006).

After 1980 proton pump inhibitor (PPI) medications were marketed, which today are the mainstay treatment of GERD (Gustavsson et al., 1983; van Pinxteren et al., 2004).

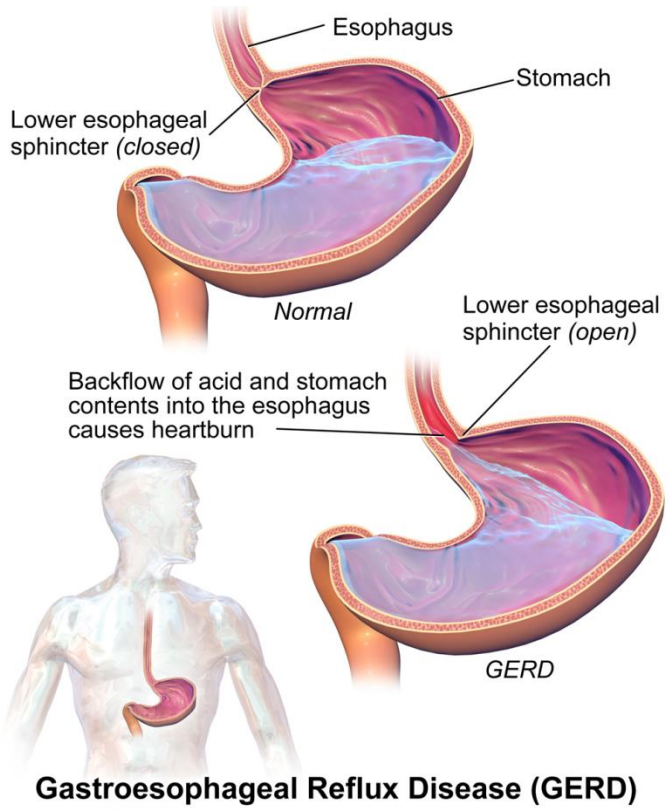


Figure 1. Mechanism of gastroesophageal reflux.

1.1.1 Diagnosis

Even though the mechanisms and classical symptoms of reflux episodes are relatively well known, definitive diagnostic criteria for GERD have been difficult to establish. This is in part because heartburn and regurgitation are very common in the general population, often mild and sporadic, and reflux episodes can be measured to some degree in practically all healthy individuals (Zerbib *et al.*, 2005). Consequently, the Montreal definition of GERD defines GERD as a condition that develops when reflux of stomach contents causes troublesome symptoms and/or complications (Vakil et al., 2006). The diagnosis is therefore in most cases entirely based on subjective findings. However, it should be noted that if complications of GER arise, such as esophagitis, the diagnosis can be made in the absence of symptoms. This is important as a number of subjects with esophagitis do not have significant GERD symptoms (Dent et al., 2012).

Many questionnaires have been created to aid the diagnosis, evaluate treatment effect and for study purposes. These vary significantly in their focus and extensiveness (Vakil et al., 2013). Some include only questions about the cardinal symptoms of heartburn and regurgitation, whereas others include

all possible GER symptoms, such as epigastric or chest pain, and even extraesophageal symptoms such as cough and hoarseness (Rothman *et al.*, 2001; Shaw *et al.*, 2001). It is beyond the scope of this dissertation to discuss these questionnaires in detail, but a few aspects deserve to be mentioned. First, a recent review of GERD questionnaires found that none of the available questionnaires fulfill the current clinical or regulatory requirements put forth by the US Food and Drug Administration or the European Medicines Agency (Vakil et al., 2013). Further development of these questionnaires is therefore necessary. Second, the questionnaires were created and validated for different patient groups and outcomes, and so the choice of questionnaire needs to be undertaken with regard to the clinical scenario.

1.1.2 Nocturnal GER

For various reasons, nocturnal (n)GER has been proposed to define a specific condition, as reflux episodes during the night have a different character than those during daytime (Orr, 2010). In healthy individuals, reflux episodes are common when upright, but very infrequent in the supine position (Zerbib et al., 2005). If a reflux episode occurs during sleep, the gastric contents can reach higher up into the esophagus and are therefore more likely to reach the airways (Orr et al., 2000). Interestingly, this is more dependent on the sleeping state rather than the supine position. Additionally, reflux episodes last longer during sleep, resulting in a longer continuous acid exposure with each episode (Campos et al., 1999; Orr et al., 1984). This correlates well with studies on reflux esophagitis subjects, where those with mild esophagitis had predominantly daytime reflux and virtually nonexistent nocturnal reflux, but those with more severe esophagitis had predominantly nocturnal reflux (Adachi et al., 2001; Kindt et al., 2011). In light of these findings, nGER has been hypothesized to be more harmful to the airways than GER in general (Orr, 2010).

1.1.3 Laryngopharyngeal reflux

One of the extra-esophageal manifestations of GER is laryngopharyngeal reflux (LPR), where the refluxate reaches the larynx and causes local inflammation. The predominant symptoms are throat clearing, cough, globus sensation, and hoarseness. In addition to the symptoms, inspection of the larynx is central to the diagnosis (Figure 2) (Ford, 2005). However, the inter-rater variability of laryngoscopy scorings varies significantly between studies, from being poor to excellent, possibly explained by some studies giving the laryngologists training and some not, as well as different study populations (Belafsky et al., 2001; Branski et al., 2002). Additionally, most of these signs are nonspecific and can be found to some degree in healthy adults (Campagnolo et al., 2014; Hicks et al., 2002). A specific score to reliably evaluate the fiberscopic findings in LPR, and to monitor changes in the disease, was created in 2001. It grades eight findings in laryngeal examination by fiberoptic laryngoscopy suggestive of LPR, giving a score from 0 to 26, and is called the reflux finding score (RFS) (Belafsky et al., 2001). This method seems to have good reliability, but is not specific (de

Bortoli et al., 2012; Payne et al., 2006). The diagnosis of LPR is therefore a difficult one, as there is no gold standard for diagnosis.

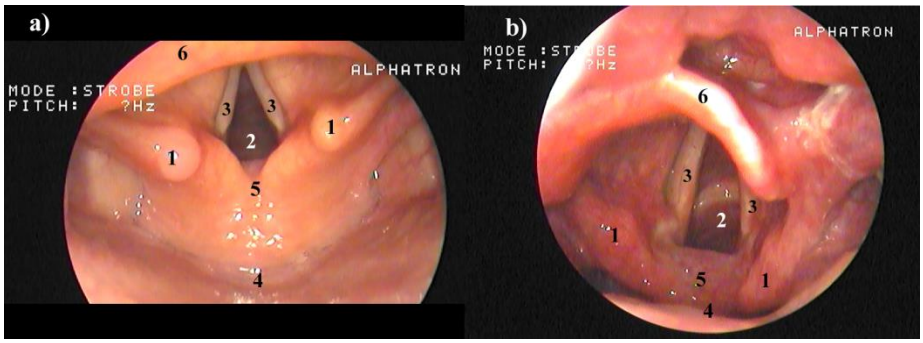


Figure 2. Pictures from a laryngoscopy. 1 - Arytenoid cartilage; 2 - Trachea; 3 - Vocal cords; 4 - Esophageal opening; 5 - Posterior commissure; 6 - Epiglottis. a) Normal larynx with normal mucus membranes, no edema. b) Larynx with inflammation, general erythema of the mucus membranes including over arytenoids, vocal cords and the epiglottis. Posterior commissure hypertrophy can also be seen.

1.1.4 24 hour esophageal multichannel pH-impedance monitoring

In 1974, 24 hour esophageal pH monitoring was first reported as a tool to objectively measure acid reflux episodes (Demeester *et al.*, 1974; Johnson & Demeester, 1974). A drop in esophageal pH below 4 was defined as an acid reflux episode. The key normal values were initially defined as less than 50 episodes per 24 hours, or total time with pH below 4 less than 4.2% of the measurement time. Recent normal values are rather similar, with normal values described as less than 50 reflux episodes per 24 hours, or total time with pH below 4 less than 2.0% of the measurement time (Zerbib et al., 2005).

However, as this method did not identify non-acid reflux episodes, a new and more sensitive method was added, multichannel impedance monitoring (Nguyen *et al.*, 1999; Silny, 1991). This method can accurately measure non-acid reflux episodes, even among patients on acid-suppressive therapy (Sifrim *et al.*, 2004). However, the clinical value of this addition is somewhat debated (Patel et al., 2014).

Studies on outcomes in GERD patients are primarily based on acid parameters as impedance monitoring is relatively new, and the pH-based parameters are better predictors of response to PPI treatment (Pandolfino & Vela, 2009; Patel *et al.*, 2014; Sifrim & Zerbib, 2012). However, this could also reflect the fact that PPI treatment only suppresses the acid component of the refluxate, and therefore likely only affects symptoms related to acid reflux episodes. In support of this, a study on GERD patients refractory to PPI treatment showed that 16 of 17 patients with a positive 24 hour esophageal multichannel pH-impedance (24h MII-pH) monitoring became asymptomatic after surgical treatment. Most of these patients had a more significant non-

acid reflux (Mainie *et al.*, 2006). Non-acid reflux seems to be associated with extra-esophageal symptoms, which also are often refractory to PPI treatment (Kawamura *et al.*, 2004; Tutuian *et al.*, 2006).

As clinical guidelines recommend empirical PPI treatment as the first step for suspected GERD, a 24h MII-pH monitoring is mostly done on therapy-resistant GERD patients (Hemmink *et al.*, 2008; Vaezi, 2011). Therefore, few data exist on how much PPI treatment in general affects reflux episodes. Additionally, some studies suggest it is rather the results of the pH monitoring than the results of the impedance monitoring that predict symptom response on PPI treatment (Patel et al., 2014). Studies on therapy-resistant GERD patients suggest that the number of reflux episodes are not affected by PPI therapy *per se* (Clayton et al., 2012; Hemmink et al., 2008). To our knowledge, no prospective studies have been performed on the effects of PPI therapy on 24h MII-pH monitoring in a PPI-naive GERD patient group.

Of special interest in the context of this dissertation are the results of 24h MII-pH measurements among chronic cough patients. These patients do often have a physiological magnitude of reflux episodes, but a clear association between reflux episodes and cough episodes (Blondeau et al., 2007; Smith et al., 2010). One study found that chronic cough patients with pathological acid exposure time or pathological impedance baseline were more likely to respond to PPI (Ribolsi et al., 2014). This study was not placebo-controlled. Taken together, even though the 24h MII-pH monitoring is still the gold standard for the measurement of GER, it is unclear which factors of the reflux are clinically most important.

1.1.5 Histopathology

GER can lead to significant changes in the esophageal mucosa. Among individuals with GERD, somewhat less than 50% have macroscopically visible reflux esophagitis. GER can in turn lead to Barrett´s esophagus and esophageal cancer (Thomson et al., 2003; Vakil et al., 2006). Interestingly, even patients without visible esophagitis can have changes in the esophageal mucosa. A study on biopsies from a macroscopically normal esophageal epithelium in GERD patients have revealed that the intercellular spaces in the epithelium were dilated (Jovov et al., 2011). This was most likely caused by cleavage of the junctional protein E-cadherin, a protein important to the barrier function of the esophageal epithelium. This protein, however, is not cleaved in eosinophilic esophagitis, a condition where the intercellular spaces are also dilated, suggesting the cleavage of E-cadherin is caused specifically by the refluxate (Jovov *et al.*, 2011). A relatively small study on children with GERD and GER-related cough found a similar pattern of dilated intercellular spaces in their distal esophagus which was not related to 24h MII-pH monitoring parameters (Borrelli et al., 2014).

The presence of pepsin in laryngeal epithelium has been found to promote proliferation of the epithelial cells.(Johnston *et al.*, 2012) A study on laryngeal biopsies from 15 patients with laryngopharyngeal reflux showed a high degree of pepsin present intracellularly, which most likely came from

endocytosed particles of the refluxate. In comparison, only a minority of 21 healthy controls had weakly pepsin-positive biopsies. Interestingly, no clear difference between acid and non-acid reflux was found. (Jiang *et al.*, 2011; Johnston *et al.*, 2007).

1.2 Respiratory symptoms in GER

An association exists between GER and diverse airway symptoms, as well as asthma diagnosis and chronic cough (Allen & Newhouse, 1984; Gislason et al., 2002; Janson et al., 1995; Ludviksdottir et al., 1996; Sontag, 2000).

The association between GER and chronic cough has been much studied. Several studies have suggested that GER may be a contributing factor in patients with chronic cough, although the causality has been difficult to prove (Morice et al., 2014; Vakil et al., 2006). In recent years, the concept of a cough hypersensitivity syndrome has become the dominant theory explaining chronic cough. In short, the theory suggests that chronic cough arises from a hypersensitivity of airway sensory nerves (Morice et al., 2014; Qiu et al., 2011). The nerves may be hypersensitive to different stimuli in different patients. GER fits well into this theory as a stimulus to cough, as explained below under proposed pathogenetic mechanisms.

The association between nGER and asthma is of special interest, as asthma is a common disease with a significant subgroup of severe or difficult to treat asthma. Even though most asthma patients can be effectively treated with current treatment options, some patients do not respond well to these treatments for unknown reasons (Chung et al., 2014; Hekking et al., 2015; Lotvall et al., 2009; Mincheva et al., 2014). These difficult to treat asthma patients often have significant comorbidities, especially when treatment with oral corticosteroids is required (Sweeney et al., 2016). Asthma has been linked to GER, but a causal association has not been clearly established. A systematic review of studies on the association between asthma and GER, with special focus on the direction of the association, indicated a significant association between the two but insufficient data on the direction of causality (Havemann et al., 2007). It is therefore important to assess whether GER may be a provocative or causative factor in selected asthma patients, and by which mechanisms. That may ultimately lead us to how to optimally treat these patients and gain better asthma control.

Studies on chronic obstructive pulmonary disease (COPD) exacerbations and GER have shown a significant association between the two, suggesting that GER might play a role in COPD exacerbations (Terada et al., 2008). A recent prospective study on risk factors for exacerbations among 2113 COPD patients found GER to be a significant risk factor for exacerbations (Busch et al., 2016). Of interest, acid suppressive therapy has been shown to be highly used or even overused in hospitalized COPD patients, albeit often because of concomitant corticosteroid therapy (Niklasson et al., 2003). Observational data do not support the use of acid suppressive therapy for COPD exacerbations (Baumeler et al., 2016). On the other hand, one small single-blind interventional study found treatment with the PPI lansoprazole to reduce

exacerbations among COPD patients without GERD (Sasaki et al., 2009). These findings need to be studied further, as the association between GER and exacerbations has a potentially high clinical relevance.

GER has also been shown to be associated with the development of bronchiolitis obliterans syndrome in lung transplant patients, a sign transplant rejection. This is likely mediated through aspiration of gastric contents, as discussed further in the next section (Blondeau et al., 2008).

Variable results are found on lung function among GER subjects. A study on 32 otherwise healthy GER subjects, compared with controls, showed a significantly lowered forced expiratory volume in one second (FEV1) and diffusion capacity of carbon monoxide (DLCO) (Mise *et al.*, 2010). However, a population based study found no association between nGER and FEV1, but rather increased peak flow variability (Gislason et al., 2002). This may be due to the definition of GER in the various studies, as the former study included GER subjects with a more significant disease level.

1.2.1 Proposed pathogenic mechanisms

Currently, there are two main theories on how GER might induce respiratory symptoms. One proposes that repeated microaspiration of gastric contents to the airways via proximal reflux causes airway inflammation (the reflux theory). The other proposes that reflux of gastric contents to the distal esophagus triggers a vagally mediated nerve reflex that causes bronchoconstriction (the reflex theory) (Figure 3).

These two theories have both been supported by research findings (Amarasiri et al., 2013; Bathoorn et al., 2011; Blondeau et al., 2008). Each mechanism is likely associated with a somewhat different symptomatology. The reflux theory is probably the main explanation of the association between GER and rejection of lung transplants (Blondeau et al., 2008). A dose response has been shown between the magnitude of bile acids and pepsin in bronchoalveolar lavage (BAL) and a shortened time to rejection of a transplanted lung (D'Ovidio et al., 2005). On the other hand, the reflex theory is probably best associated with chronic cough. Studies have found neuroinflammatory markers to be elevated among subjects with GER and chronic cough, but not markers of aspiration (Grabowski et al., 2011; Patterson et al., 2007; Qiu et al., 2011). Among asthma patients, GER is associated both with signs of increased sensory nerve activation as well as aspiration, indicating that both mechanisms might play a role in the association between GER and asthma (Patterson et al., 2007; Perng et al., 2007). One study performed an acid perfusion test on the distal esophagus of 40 stable asthma patients and found that the acid perfusion induced bronchoconstriction (Amarasiri et al., 2013).

Finally, the significance of the pulmonary microbiota and its association with respiratory diseases has recently become of special interest (Yadava et al., 2015). The possible effects of aspiration of gastrointestinal microbiota remain to be studied further (Madan et al., 2012; Willner et al., 2013).

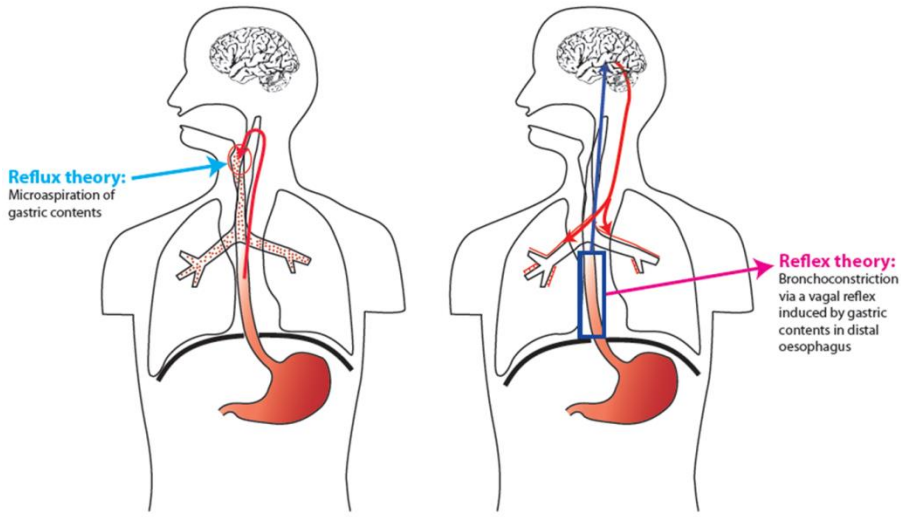


Figure 3. Two theories on how gastroesophageal reflux may cause respiratory symptoms.

1.3 Sleep-disordered breathing

1.3.1 Obstructive sleep apnea

Sleep-disordered breathing (SDB) is a broad term for many nocturnal respiratory syndromes. It includes primary snoring, upper airway resistance syndrome, obstructive sleep apnea-hypopnea (OSA), and obesity hypoventilation syndrome. OSA is a condition caused by repeated cessation of breathing (apneas) or declines in breathing (hypopneas) during sleep due to obstruction of the upper airway. The cardinal symptoms other than apneas during sleep are snoring and daytime sleepiness. OSA is currently diagnosed by a sleep study where apneas and hypopneas are measured, and an apnea-hypopnea index (AHI) is calculated as events/hour. The classification defines an AHI of 5-15 as a mild disease, 15-30 moderate disease, and 30 or more as a severe disease (Epstein et al., 2009).

A positive association between OSA and GER has been known for many years (Samelson, 1989). Sleep disturbances are common among patients with reflux esophagitis and have been shown to decrease with PPI treatment (Kindt et al., 2011). In patients where reflux episodes trigger apneas, esophagitis is common and their AHI decreases after PPI treatment (Ermis *et al.*, 2011). However, reflux-induced apneas are uncommon, which is likely the reason why PPI treatment does not affect the AHI for most OSA patients (Gerson & Fass, 2009; Orr et al., 2014; Orr et al., 2009; Yang et al., 2013).

In this aspect, reflux affecting the larynx (LPR) may possibly have a stronger association with OSA than patients with GER. One small study found that OSA patients with LPR had more spontaneous arousals than OSA patients with GER (Suzuki et al., 2010). Laryngeal inflammation has also been found

to be prevalent among OSA patients and correlate with apnea severity (Payne et al., 2006). However, further studies are needed to examine this relationship.

On the other hand, OSA has been hypothesized to lead to nGER. Recent studies have shown that the lower esophageal sphincter contracts during apneic episodes and thereby inhibits gastric acid reflux (Berg et al., 2004; Kuribayashi et al., 2010; Shepherd et al., 2011). This repeated strain on the lower esophageal sphincter caused by decreased intrathoracic pressure during obstructive events may cause “fatigue” in the sphincter, leading to more reflux events (Kuribayashi et al., 2010; Shepherd et al., 2011). Treatment with positive airway pressure (PAP) leads to increased pressure in the lower esophageal sphincter (Shepherd et al., 2007), which might explain why nGER symptoms often decrease among OSA patients when treated with PAP (Green et al., 2003; Kerr et al., 1992).

The nature of this association is unclear, however, and it has been debated whether there is a causal relationship or co-occurrence because of shared risk factors, of which obesity is the most important one (Basoglu et al., 2015; Kahrilas, 2010). It is therefore central to account for the effects of obesity in studies on nGER and OSA.

1.3.2 Partial upper airway obstruction (snoring)

The primary measurement used to define and grade OSA is the AHI, as mentioned above. However, a recent study on a general population clearly showed a poor correlation between AHI and daytime sleepiness, the cardinal symptom of the OSA syndrome. Only those with the highest AHI, 30 or more, did have significantly increased daytime sleepiness (Arnardottir et al., 2015a). This is supported by previous studies with similar findings, where snoring has even been shown to be better associated with daytime sleepiness than AHI (Svensson et al., 2008; Tam et al., 2014). This might be explained by episodes of increased upper airway resistance which cause increased respiratory effort followed by arousals, which in turn lead to daytime sleepiness. These episodes can be alleviated by CPAP, just as apneic episodes (Guilleminault et al., 1993). Also, snoring has been shown to cause local neuronal lesions in the pharynx, and may cause carotid atherosclerosis (Friberg et al., 1998; Lee et al., 2008). Therefore, using more markers than just AHI seems to be important when studying sleep-disordered breathing.

Many of these studies have relied on subjectively reported snoring for defining snorers. A few methods exist to measure snoring, but the quality varies significantly. A study comparing these methods found audio-based measurements to be most reliable to detect snoring (Arnardottir et al., 2015b). Further studies are needed to determine which dB range and frequencies are most harmful.

1.4 Respiratory biomarkers

In recent years, there has been increasing interest in studies on respiratory

biomarkers collected non-invasively, mostly from sputum or exhaled air samples. Such samples make it possible to study local reactions in a much wider population than invasive samples, such as bronchoalveolar lavage. Regarding GER and respiratory diseases, there is a need for a diagnostic test that can discriminate between respiratory diseases caused by GER or by other causes. Such a test should ideally be non-invasive, with a high positive predictive value, and react to effective treatment. To date, no such specific diagnostic test is available, but exhaled biomarkers hold certain promise and need to be studied further in this context.

Respiratory biomarkers seem to differ between children and adults. For example, pepsin seems to be more common in induced sputum among healthy children than healthy adults (Decalmer et al., 2012; Ervine et al., 2009).

A few studies have evaluated pepsin levels in biosamples from the respiratory tract in subjects with GER. Studies on chronic cough have not found pepsin to be elevated among GER subjects (Decalmer et al., 2012; Grabowski et al., 2011). Studies on GER in asthma and COPD showed conflicting results regarding pepsin in respiratory samples (Rosen et al., 2012; Timms et al., 2012). However, pepsin did correlate with reflux and chronic rejection in lung transplant patients, albeit not as strongly as bile acids (Blondeau et al., 2008; Reder et al., 2014). A limitation in some of these studies was that pepsin was only measured as present or absent. This may be a confounding factor, as pepsinogen has been found to be produced in alveolar type 2 cells in the lungs (Elabiad & Zhang, 2011; Gerson et al., 2008). Therefore, pepsin can not be at present considered as pathognomonic for GER-related aspirations, but further studies are needed as well as more standardized quantitative measurements.

The lipid laden macrophage index (LLMI) in BAL was previously considered a marker of aspiration (Corwin & Irwin, 1985). However, studies on LLMI in BAL samples have shown conflicting results. LLMI has been found to be elevated in pulmonary diseases with no evidence of aspiration and does not correlate with any parameters on a 24 hour esophageal pH-monitoring in children (Kitz et al., 2012; Knauer-Fischer & Ratjen, 1999). Therefore, the LLMI in BAL seems to be unhelpful in diagnosing GER-related respiratory diseases.

As discussed above, GER probably is associated with different respiratory diseases through different mechanisms. This is reflected in different biochemical findings. In chronic cough, common markers of gastric fluid aspiration such as pepsin and bile acids are usually not elevated (Decalmer et al., 2012; Grabowski et al., 2011; Patterson et al., 2007; Qiu et al., 2011; Starosta et al., 2007). However, tachykinins such as substance P and neurokinin A associate with GER in chronic cough subjects, indicating a vagally-mediated bronchoconstrictive reflex (Patterson et al., 2007; Qiu et al., 2011). In contrast, lung transplant patients with GER have significantly elevated levels of pepsin and bile acids, quantitatively, indicating gastric fluid aspiration as a predominant causative factor (D'Ovidio et al., 2005; Mertens et al., 2011). A study on sputum and EBC biomarkers among GER and

asthma subjects showed that IL-6 and 8-isoprostane were elevated among GER subjects irrespective of asthma status (Carpagnano et al., 2006). These findings indicate that the different pathogenic mechanisms are reflected in the respiratory biomarkers.

A few methodological aspects need to be mentioned regarding non-invasive biosamples from exhaled air. EBC measurements have shown to have little reproducibility and are poorly standardized, as instruments and collection techniques vary, making their usefulness currently limited. For the application of EBC to become more successful, collection methods and biomarker analyses in EBC samples need to become more standardized (Davis et al., 2010; Rosias, 2012). Sputum samples are often contaminated by saliva, and only reflect the proximal airways. A new method to non-invasively collect undiluted samples from the small airways' lining fluid, called particles in exhaled air (PExA^{TM}), has recently been described, but to date not studied in GER and respiratory diseases (Bredberg et al., 2012).

In summary, subjects with GER and respiratory diseases seem to have a different biochemical profile than similar subjects without GER. Biomarkers which have been shown to associate with GER in respiratory diseases are summarized in Figure 4. Inflammatory markers differ in asthmatics based on GER status, lung trantsplanted subjects with GER have elevated bile acids in BAL samples, and tachykinins are elevated in GER-related chronic cough. However, most of these studies are small and few address the same subject, which makes it hard to draw definite conclusions.

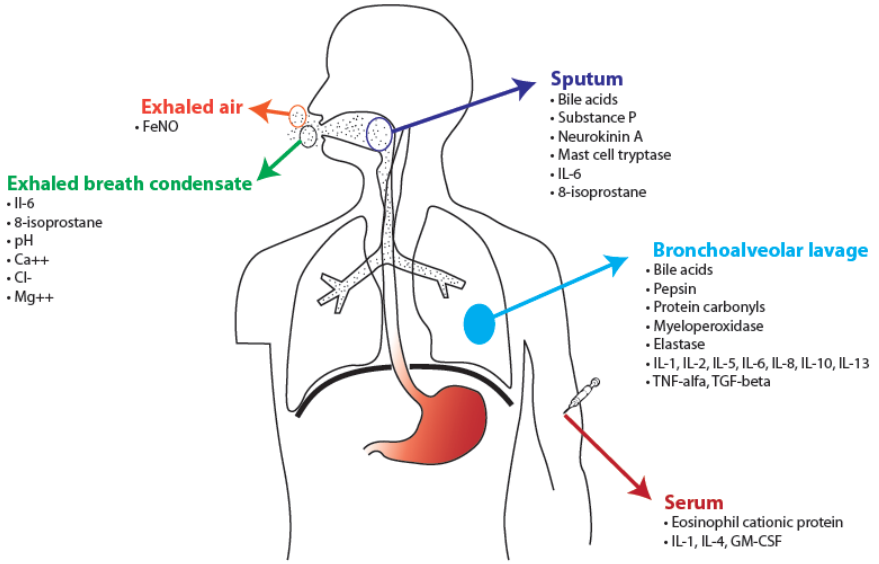


Figure 4. Summary of biomarkers shown to have an association with gastroesophageal reflux in respiratory illnesses.

2 Aims

The overall aim of this doctoral dissertation was to investigate the associations of nGER with respiratory illnesses and sleep-disordered breathing (SDB), primarily OSA. The studies planned are based on epidemiological data from population based studies and also further investigations on subpopulations. The specific research questions and hypotheses were:

1. Is nGER associated with respiratory symptoms, changes in lung function and symptoms of SDB? (Papers I and II)
2. Does nGER precede respiratory symptoms and symptoms of SDB in a general population? Is persistency of nGER symptoms over long time periods important in this context? (Papers II and III)

Hypothesis 1: Symptomatic nGER can induce respiratory symptoms and symptoms of SDB, especially if persistent over long periods of time.

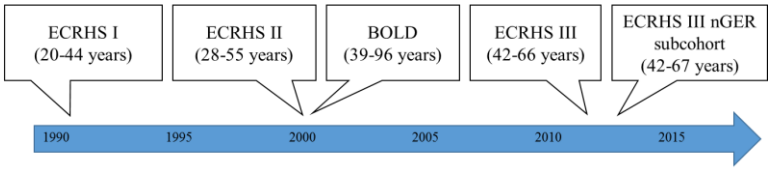
3. Is nGER associated with elevated inflammatory biomarkers in exhaled air? Are inflammatory biomarkers or pepsin in exhaled air associated with respiratory symptoms and SDB in nGER patients? (Paper IV)
4. Does SDB affect the association between nGER and respiratory symptoms? (Paper IV)

Hypothesis 2: OSA is worse in subjects with nGER because of inflammation in the proximal airways, making the airways more susceptible to collapsing during the night and thereby cause obstructive apneas. Gastric contents are intermittently aspirated in nGER, causing airway inflammation and respiratory symptoms. Pepsin in exhaled air can identify subjects where gastric contents have reached the airways.

3 Materials and methods

3.1 Study cohorts

The research papers in this dissertation were based on results from two different study cohorts. Paper I was based on the burden of obstructive lung diseases initiative (BOLD) cohort in Iceland and Sweden; paper II on the European community respiratory health survey (ECRHS) I and II cohort in Iceland, Sweden and Belgium; and papers III and IV on the ECRHS III cohort in Iceland (Figure 5, Table 1). The National Bioethics Committee and the Data Protection Authority of Iceland gave consent for all three study cohorts. The Swedish Research Council gave their consent for the BOLD study cohort. The Regional Ethical Committee of Uppsala University, Sweden, and the Advisory Committee for Medical Ethics of the University of Antwerp, Belgium, gave their consent for the ECRHS study cohort. Written consent was obtained from all research subjects.



Reykjavik	890 invited, 69% fully participated	608 invited, 82% fully participated	939 invited, 81% fully participated	468 invited, 90% fully participated	145 invited, 83% fully participated
Uppsala	921 invited, 79% fully participated	719 invited, 77% fully participated	998 invited, 62% fully participated		
Gothenburg	998 invited, 54% fully participated	535 invited, 71% fully participated			
Antwerp	1895 invited, 41% fully participated	778 invited, 42% fully participated			

Figure 5. Timeline for the studies used in this dissertation. The age span of the participants is noted in parentheses under the study name. The participation rate was calculated from the number of subjects who participated in all aspects of the studies.

3.1.1 BOLD cohort

The BOLD initiative was a multicenter international study aiming to estimate the burden of COPD worldwide (see www.boldstudy.org) (Buist et al., 2007). The cohort was a random sample from the general population in Reykjavik, Iceland, and Uppsala, Sweden, aged 40 years and over (Benediktsdottir et al., 2010). The study was mostly conducted in 2005 in these centers. Briefly, they participated in an examination that included a structured interview, medical examination, blood sample and spirometry. In Iceland, 939 subjects were randomly selected to participate, of which 762 subjects (81.2%) responded. In Sweden, 967 subjects were randomly selected to participate, of which 601 subjects (62.2%) responded. Of these 1,363 subjects, 1,325 had sufficient data for the study analysis.

3.1.2 ECRHS cohort

The ECRHS I - III was a 20 year prospective, multicenter, population-based cohort study performed in 11 European countries (see www.ecrhs.org). The study participants were first studied in 1991-1993, then aged 20-44 years (Burney et al., 1994). They were then re-studied in 1999-2001 (Committee, 2002), and for the third time in 2011-2012, when the participants were aged 40-65 years. The investigation included a structured interview, questionnaires, spirometry, skin-prick tests, and blood samples.

Altogether 2,661 subjects participated in ECRHS I in Reykjavik, Iceland; Uppsala and Göteborg, Sweden; and Antwerp, Belgium. Of these, 2,202 were randomly selected from the general population and 459 were added because of reported asthma (Janson et al., 1996). These subjects were invited again for ECRHS II, of whom 1,761 (66%) participated.

Among the 522 subjects from ECRHS I contacted for ECHRS III in Iceland, a total of 455 (87%) participated (Arnardottir et al., 2015a). They participated in a structured interview, answered questionnaires, underwent spirometry, measurements of height and weight, gave blood samples, and underwent a home sleep study.

3.1.2.1 ECRHS III nGER subcohort

Of the 455 participants in ECRHS III in Iceland, 82 had symptoms suggestive of nGER. These 82 subjects were invited for a second visit in 2013, of which 71 (87%) participated. Also, 63 age and gender paired controls from the ECRHS III cohort without any nGER symptoms were invited, of whom 49 (78%) participated. Those with a persistent symptom report comprised the cohort, altogether 90 subjects (see section 3.2.2 for further description).

During the second visit all subjects participated in a structured medical interview, answered a modified reflux disease questionnaire (RDQ), were weighed again, and examined with a laryngoscopy. Exhaled breath condensate (EBC), particles in exhaled air (PEx) and blood were sampled. Nitric oxide in exhaled air (FeNO) was measured. A subgroup underwent a 24 hour esophageal pH-impedance measurement (24h MII-pH).

Table 1. Cohorts studied in this dissertation. Abbreviations: BOLD, Burden of obstructive lung disease initiative; ECRHS, European community respiratory health survey.

	BOLD cohort (n = 1,325)	ECRHS I and II cohort (n = 1,761)	ECRHS III nGER subcohort (n = 90)
Paper I	X		
Paper II		X	
Paper III			X
Paper IV			X

3.2 Questionnaires and interviews

3.2.1 nGER questionnaire

In studies I and II, we defined nGER using a single question on the frequency of heartburn and regurgitation (see below under nGER definition). As this single question approach was not well validated, we chose in our following studies to use a better validated questionnaire for the definition of our study groups.

Of the many questionnaires created for evaluating GER, only a few have been validated for both evaluative and diagnostic purposes, and used in clinical trials (Vakil et al., 2013). For studies III and IV, we wanted to use a simple questionnaire with evaluation of the most cardinal symptoms of GER, namely heartburn and regurgitation, as this has been recommended for studies on extra-esophageal manifestations of GER (Vakil et al., 2006). Additionally, we wanted to divide symptoms into daytime and nocturnal symptoms. Unfortunately, none of the available questionnaires fulfilled our criteria, but two were near, namely the RDQ and the nocturnal gastoesophageal reflux disease symptom severity and impact questionnaire (N-GSSIQ). The former was validated for evaluative and diagnostic purposes in a general population and asked only about the cardinal symptoms of heartburn and regurgitation, but did not ask about daytime or nocturnal symptoms. The latter was only evaluative, solely focused on nocturnal symptoms, and had extensive questions on symptoms other than heartburn or regurgitation (Shaw et al., 2001; Spiegel et al., 2010). We therefore chose to adapt the RDQ rather than the N-GSSIQ to our needs.

The RDQ questionnaire had not previously been translated into Icelandic. We therefore first translated it from English to Icelandic, and then validated it via back-translation. We used only the heartburn and regurgitation dimensions of the questionnaire, as only these two dimensions have shown an association to GER, and not the dyspepsia dimension (Shaw et al., 2001). The questions from these two dimensions were posed twice, once specifically for daytime symptoms and once specifically for nocturnal symptoms. The questionnaire covers symptoms in the preceding four weeks.

A symptom score was calculated from the RDQ for daytime and nocturnal symptoms by adding together the points for both frequency and severity of the respective GER symptoms (altogether eight variables) and then dividing by eight, giving points on a scale from 0 to 5.

3.2.2 nGER definition

In papers I and II, the definition of nGER was based on a single question on the frequency of heartburn or regurgitation after going to bed in the preceding four weeks. For the cross-sectional analysis in paper I, nGER was defined as experiencing symptoms once a week or more, and those without symptoms were further divided into whether they were on GER medication or without GER medication. For the longitudinal analysis in paper II, nGER was defined

as experiencing symptoms less than once a week or more, and then grouped into non-symptomatic, symptomatic at baseline or follow-up, and persistently symptomatic. Those persistently symptomatic were of special interest.

The definition of nGER in the ECRHS nGER subcohort, used in papers III and IV, was based on the modified version of the RDQ, as described above. Those reporting any nocturnal GER symptoms on the first (2012) and second visit (2013) were defined as having nGER (n = 48). Those without nocturnal GER symptoms on the first and second visits were defined as controls (n = 42). Those with nocturnal GER symptoms on only one of the visits were excluded in paper IV, but included for sensitivity analysis in paper III (Figure 6).

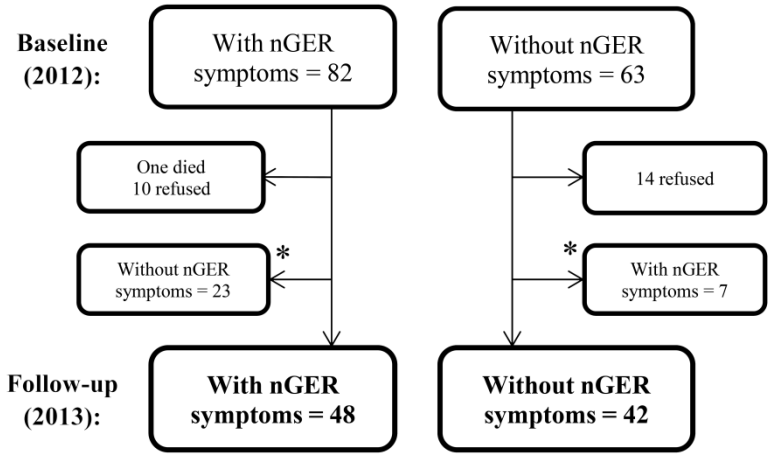


Figure 6. A flow diagram of selection of cases and controls for the ECRHS III nGER subcohort. At the top are subjects at the time of first visit in the ECRHS III study, and at the bottom at follow-up around 5-8 months later. The groups in bold were used for further analysis in papers III and IV: subjects who were symptomatic at baseline and follow-up (persistent nGER), and subjects who were asymptomatic at baseline and follow-up (controls). The groups marked with an asterix were only included for sensitivity analysis in paper III.

3.2.3 Symptom analysis

Symptoms were analyzed in all cohorts by questionnaires and structured medical interviews. The symptom assessments differed between the studies and are therefore best described separately for each study.

3.2.3.1 BOLD

All participants visited the study clinic where they answered a questionnaire posed by trained and certified staff. The questionnaire regarded respiratory, OSA and nGER symptoms, smoking, health status, medication use and other health related issues (Benediktsdottir et al., 2010; Buist et al., 2007; Partinen & Gislason, 1995). The questions on respiratory symptoms asked whether

they had experienced symptoms such as cough, phlegm and wheeze (Buist et al., 2007). The participants were divided into smokers, ex-smokers and never-smokers by answers to smoking history questions. The OSA-related questions included the Epworth sleepiness scale (ESS) and questions from the Basic Nordic Sleep Questionnaire, such as self-reported snoring, apneas and daytime sleepiness (Benediktsdottir et al., 2010; Partinen & Gislason, 1995).

Those reporting either coughing most days for up to three months each year, having phlegm most days for up to three months each year, any wheeze for the last 12 months or shortness of breath when hurrying were classified as having “any respiratory symptom”. Those with observed snoring or daytime sleepiness more than twice a week, or with observed apneas once a week or more, were classified as having “any OSA symptom”.

3.2.3.2 ECRHS I and II

The participants answered detailed questionnaires, including questions on respiratory, OSA and nGER symptoms, smoking, health status, medication use and other health related issues (for ECRHS questionnaires, see http://www.ecrhs.org/) (Burney et al., 1994).

Subjects were considered to have asthma if they reported having been diagnosed with asthma by a physician plus having asthma-related symptoms in the last 12 months (Janson et al., 2005). Yes/no-questions were posed about respiratory symptoms at any time in the last 12 months: wheezing, nocturnal chest tightness, shortness of breath at rest and after exercise, nocturnal shortness of breath and nocturnal cough. Subjects who had had any of these respiratory symptoms in the last 12 months were additionally classified as having “any respiratory symptom”. Participants defined with asthma, or reporting a particular symptom at follow-up but not at baseline, were defined as having an onset of asthma or respiratory symptoms during the study period (Gunnbjornsdottir et al., 2004).

Symptoms of OSA were estimated by a questionnaire and defined as self-reported snoring, apneas, or daytime sleepiness. The same questions were used at baseline and follow-up. _ENREF_15Those reporting observed snoring or daytime sleepiness more than twice a week, or observed apneas once a week or more, were considered to have the corresponding symptom. Those with any of the above-mentioned symptoms were additionally classified as having “any OSA symptom”. For a more OSA-specific analysis of these symptoms, those with new snoring and/or apnea plus new daytime sleepiness were also analyzed together.

Participants also answered the ESS on participation in ECRHS II (Benediktsdottir et al., 2010). A score of 10 or higher was considered as significant daytime sleepiness. ESS was not used when analyzing onset of OSA symptoms as it was not available from ECRHS I.

Smoking history was investigated by asking subjects at baseline and follow-up whether they were current smokers, ex-smokers or never-smokers. Based

on this information the subjects were classified into Never-smoker, Ex-smoker, Quitter, and Smoker.

3.2.3.3 ECRHS III nGER subcohort

A structured medical interview was performed to collect information on health status changes since the last visit (ECRHS III baseline visit in 2012). The interview asked specifically about changes in general health status, changes in medication, hospital stays, and a doctor's diagnosis of high blood pressure, diabetes, asthma, COPD, heart attack, heart failure and stroke. The interview also contained a questionnaire on recent respiratory and OSA symptoms using selected questions from the previous visit's questionnaire. The ESS questionnaire was only answered on the baseline visit.

In total, seven cases and seven controls reported changes in health status at follow-up since their baseline visit. In the case group, health changes included diagnosis of hemochromatosis, removal of a liver cyst, lumbago, etc. In the control group, health changes included coronary artery disease, discus prolapse, hypothyroidism, candida esophagitis, etc. Altogether 14 cases had changes in their medical treatment, of whom one discontinued PPI treatment, one started PPI treatment and four had changes in PPI treatment. Among controls, six had changes in their medical treatment, none of which regarded PPI.

The questions on respiratory symptoms were the same as in ECRHS I and II. For analysis purposes in paper IV, the symptoms were grouped into asthma symptoms (wheeze, chest tightness, breathlessness at rest, breathlessness after exercise, nocturnal cough, nocturnal attacks of breathlessness) and bronchitis symptoms (cough or phlegm without ongoing respiratory infection).

In paper IV, an exacerbation of respiratory symptoms was defined as a temporary marked increase in respiratory symptoms: cough, phlegm and shortness of breath._ENREF_13 Frequent exacerbations were defined as at least two exacerbations in the previous 12 months. Current asthma was defined as having doctor diagnosed asthma as well as either experienced asthma symptoms in the previous 12 months and/or currently using any medicines for asthma (Carsin et al., 2013).

Symptoms of OSA were analyzed by a questionnaire developed by the Sleep apnea global initiative consortium (SAGIC), where participants were asked about symptoms in the previous month (Arnardottir et al., 2015a). Specifically, the questions regarded frequency of self-reported snoring and apneas on a five point scale (Never, less than once a week, 1-2 times a week, 3-4 times a week, 5-7 times a week), and how sleepy they felt during the day on a five point scale (Not at all, a little bit, moderately, quite a bit, extremely). Snoring was considered positive if reported at least three times per week, and apneas if reported at least once per week. Daytime sleepiness was considered present if subjects reported their daytime sleepiness as considerable or more.

3.3 Measurements

Spirometry was performed on all cohorts and on all visits. Home sleep study was only performed on participants in ECRHS III in Iceland. Blood samples were collected from all ECRHS cohorts. Biomarker analysis in exhaled air biosamples, laryngoscopy and 24h MII-pH were only performed on the ECRHS nGER subcohort.

3.3.1 Lung function and methacholine challenge

Trained and certified technicians performed spirometry in all studies. Maximum forced expiratory volume in one second (FEV1) and maximum forced vital capacity (FVC) were determined (Chinn et al., 2005b; Vollmer et al., 2009). FEV1/FVC was calculated from these maximum values. For the BOLD cohort and the ECRHS III nGER subcohort, the predicted values were calculated with equations from the third United States National Health and Nutrition Examination Survey (NHANES) III (Hankinson et al., 1999a). For the ECRHS I and II cohorts, the predicted values were calculated with equations from the European Coal and Steel Union (Quanjer et al., 1993).

Pre- and post-bronchodilator tests were performed on the BOLD cohort and the ECRHS III nGER subcohort, with separate measurements performed before and after ≥15 min after two puffs of salbutamol (200 µg). The pre-bronchodilator test was performed in the ECRHS I and II, and change in lung function was calculated as the change per year in percent of predicted values between the two studies.

In ECRHS I and II, a methacholine challenge was carried out using a dosimeter (Mefar, Brescia, Italy). The starting dose was 0.002 mg followed by several dose steps up to an accumulated dose of 1 mg, A change in bronchial responsiveness was expressed as a change in slope per year of follow-up (Chinn et al., 2005a). Bronchial hyperresponsiveness (BHR) was defined as a fall in FEV1 of ≥ 20% following an accumulated dose of 1 mg methacholine (Shaaban et al., 2007).

COPD was defined by lung function data as a post-bronchodilatory FEV1/FVC < 0.70 (Buist et al., 2007), or as being below the age-adjusted lower limit of normality (LLN) in post-bronchodilatory FEV1/FVC (Hankinson et al., 1999b). Subjects with COPD were grouped into stages I to IV based on FEV1/FVC and FEV1 (Pauwels et al., 2001).

The reversibility test was considered positive if FEV1 increased at least 200 ml after bronchodilation as well as increased at least 12% compared to before bronchodilation.

3.3.2 Laryngoscopy

Laryngoscopy was performed transorally with a rigid 70° borescope without using anesthesia. The larynx was recorded for a few seconds by video and then stored under an encrypted code. The videos were later scored by the reflux finding score (RFS) (Belafsky et al., 2001) by three otolaryngologists,

blinded from each other and other study results. The average scorings were used for analysis. Those with a RFS of more than seven were classified as having laryngopharyngeal reflux.

3.3.3 Esophageal 24-hour multichannel intraluminal impedance and pH monitoring

In a subgroup of participants, an esophageal 24h MII-pH monitoring was performed according to a previously described method (Conchillo et al., 2007). Briefly, a MII-pH catheter (ComforTec Z/pH probe ZAN-BS-01, Sandhill scientific, Milwaukee, WI) was passed transnasally into the esophagus and positioned with the pH electrode 5 cm above the upper border of the lower esophageal sphincter. The catheter was then attached to a recording device (ZepHr z07-2000B-B, Sandhill scientific, Milwaukee, WI) and a recording performed over 24 hours. The participants registered their symptoms, both conventional (heartburn and regurgitation) and atypical (mainly cough and throat clearing) reflux symptoms.

Three participants from the control group, one with nGER symptoms only on follow-up, and 16 from the persistent nGER group underwent 24h MII-pH monitoring. Of the 16 participants from the persistent nGER group, 7 were on chronic PPI therapy, but none from the control group. From the persistent nGER group, those with more symptoms of nGER were chosen for 24h MII-pH monitoring. For scoring the measurements, reference values from Zerbib et al. were used (2005). A symptom index of 50% or more was considered positive (Tutuian et al., 2006). Mean monitoring time was 22.1 ± 1.4 hours. Because of the low number of 24h MII-pH measurements, only descriptive analysis was performed.

3.3.4 Home sleep studies

All subjects who participated in the ECRSH III in Iceland were invited for a home sleep study with a T3 device (Nox Medical, Reykjavik, Iceland), a type 3 sleep study as previously described (Arnardottir et al., 2015a). Briefly, nasal airflow was recorded through a cannula. Chest and abdominal movements were measured with respiratory inductive plethysmography belts. Pulse and oxygen desaturation were measured by a finger probe oximeter (Nonin Medical Inc., Plymouth, Minnesota). Body position, activity and audio were measured by sensors situated on the chest. The sleep studies were scored by two trained sleep technologists. Sleep studies were scored in accordance with the American Academy of Sleep Medicine 2007 manual using the accepted hypopnea classification requiring a ≥30% drop in respiratory flow for ≥10 seconds with ≥4% oxygen desaturation (Iber et al., 2007).

Snoring was measured using audio recordings from sensors on the chest. Snore events were automatically scored using the Noxturnal 4.3 scoring algorithm, with an absolute threshold for snore events set as audio volume >65 dB. In comparison to manually scored snore events, this definition has been shown to have sensitivity and a positive predictive value of 0.79 and 0.94, respectively (Arnardottir et al., 2015b). A snore index was calculated as

the number of snores per hour of sleep.

3.3.5 Blood samples

In ECRHS I and II, serum samples were collected for the measurement of total and specific serum IgE. In the ECRHS III nGER subcohort, plasma samples were collected from a peripheral vein in EDTA-treated tubes. Albumin was measured directly, but for other measurements the samples were frozen at minus 80°C until measurements were performed.

3.3.6 Biosamples from exhaled air

EBC samples were collected with ECoScreen II (FILT - Lung- and Thorax Diagnostic GmbH, Berlin, Germany) at -10°C for 15 minutes. Participants wore a nose-clip and used tidal breathing. The samples collected were immediately divided into polypropylene test tubes (Screw cap micro tube 72.694.007, Sarstedt AG & Co, Nümbrecht, Germany), 0.5 ml into each tube. Depending on the volume collected, the number of tubes varied from three to six. If any excess volume was left, it was measured and then disposed of. Into the first two test tubes, 25 µl of a mixture of protease inhibitors was added (cOmplete, Mini version 10; Roche Diagnostics GmbH, Mannheim, Germany). Nothing was added to the third test tube. Into the fourth test tube, 5 µl of a 5 mg/ml solution of butylated hydroxytoluene in ethanol was added (Sigma-Aldrich, St. Louis, MO). Other test tubes had no additives. The samples were immediately frozen at -20°C, and within four hours moved to -80°C for storage until measurements were performed.

FeNO was measured with NIOX MINO (Aerocrine AB, Solna, Sweden) according to manufacturer description (see www.niox.com). Participants were allowed a maximum of five repetitions to perform the measurement. However, more than one repetition was rarely needed and never more than three repetitions.

A method of collecting particles in exhaled air has recently been established using an instrument developed specifically for this purpose (PExA^{TM}, Sahlgrenska University Hospital, Gothenburg, Sweden) (Bredberg et al., 2012)._ENREF_18 The exhaled particles have been shown to reflect the respiratory tract lining fluid and to originate mainly from the small airways (Almstrand et al., 2010). Before sample collection start, participants breathed filtered air for two minutes tidally to avoid contamination by ambient particles. A specific breathing maneuver, allowing for airway closure and re-opening to augment the number of exhaled particles, was applied. In this maneuver, the participants exhaled to residual volume, held their breath for three seconds, then inhaled sharply up to full inspiration, and finally exhaled with a velocity between 25 and 1000 ml/s to almost full expiration. Particles were only collected from the last exhalation of this maneuver. In between these breathing maneuvers the participants breathed filtered air tidally for 30-60 seconds. The breathing maneuver was repeated until 300 ng of PEx had been collected, or until the collection had taken 30 minutes in total, whichever came first.

The total mass of the collected particles was calculated based on the number and size of the particles, assuming them to be spherical and have a density of 1000 kg/m^3.(Larsson et al., 2012) The exhaled particles were collected by impaction on a teflon filter (LCR Membrane Filter, Merck Millipore KGaA, Darmstadt, Germany), which was divided into two halves immediately after sampling, and each half was stored in a polypropylene test tube (Screw cap micro tube, Sarstedt AG & Co, Nümbrecht, Germany). The filters were immediately frozen at -20°C, and within four hours moved to -80°C until measurements were performed.

3.3.7 Biomarker measurements

In ECRHS I and II, Specific IgE was measured in serum at baseline against *Dermatophagoides pteronyssinus*, cat, birch, timothy grass and *Cladosporium herbarum,* using the Pharmacia CAP System (Pharmacia Diagnostics, Uppsala, Sweden). The detection of specific IgE of $\geq$ 0.35 kU/l was used as a definition of sensitization to a specific allergen. Atopy was defined as sensitization to at least one of the investigated allergens.

In the ECRHS III nGER subcohort, plasma albumin levels were measured using a Vitros 5.1 analyzer and Vitros MicroSlide method (Ortho-Clinical Diagnostics Inc, Rochester, NY). Albumin levels in EBC were determined by immunoturbidometry using a Virtos 5.1 analyser and reagents from Ortho-Clinical Diagnostics. Plasma high sensitivity C-reactive protein (CRP) was measured on a Cobas 411 analyzer using reagents from Roche Diagnostics GmbH, Mannheim, Germany. Interleukin 8 (IL-8) levels in plasma and EBC samples were determined using ELISA reagents (IBL International GmbH, Hamburg, Germany). Additionally, we performed ELISA measurements in EBC of pepsin (Wuhan EIAAB Science Co, Wuhan, China), 8-isoprostane (Cayman Chemical Company, Ann Arbor, MI), neurokinin A (RayBiotech Inc, Norcross, GA) and substance P (Cayman Chemical Company, Ann Arbor, MI). From the PEx samples, we registered data on the number and mass of exhaled particles and measured albumin and surfactant protein A (SP-A) as markers of pulmonary inflammation, as previously described (Larsson et al., 2012). Prior to protein assay of the PEx samples, the particles were extracted from Teflon filters using phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA), w/v, and 0.05% Tween-20, v/v. A total of 140 µl of the extraction buffer were added to each sample, followed up by 60 min shaking at 400 rpm and 37°C at a thermomixer (Thermomixer comfort, Eppendorf; Eppendorf AG, Hamburg, Germany). SP-A in PEx and plasma was quantified using human SP-A ELISA kit (BioVendor, Brno, Czech Republic) and albumin using human albumin ELISA kit (Immunology Consultants Laboratory Inc, Portland, OR), according to the manufacturer's instructions, with small modifications. The human albumin ELISA assay has no cross-reactivity to bovine albumin.

3.4 Statistical analysis

We used STATA version intercooled (Stata Corporation, College Station, TX)

for the calculations in all studies presented. Version 11.0 was used for papers I and II, and version 13.0 for papers III and IV. In all papers, a p-value of < 0.05 was considered statically significant.

Paper I: Associations between nGER status and symptoms were analyzed by a chi square test and logistic regression, while associations between nGER and continuous variables were analyzed with linear regression. Multiple logistic and linear regressions were used to estimate differences between the groups, adjust for center, age, gender, BMI and smoking history, and do interaction analyses.

Paper II: Associations were analyzed by a chi square test and linear and logistic regressions. Adjusted calculations were done by adjusting for gender, age, location, smoking history at follow-up, body mass index (BMI) at base-line and change in BMI.

Paper III: Normal distribution of continuous data was assessed both visually using histogram and with Shapiro-Wilk test (Ghasemi & Zahediasl, 2012). Intraclass correlation (ICC) for the RFS was calculated using a two-way random effects model. An average ICC was calculated, which indicates the reliability of an average score between multiple raters (Hallgren, 2012). Associations were analyzed as appropriate with student's *t*-test, chi square test, Fisher's exact test, Wilcoxon rank-sum test, and linear regressions.

Paper IV: Normal distribution of continuous data was assessed both visually using a histogram and with a Shapiro-Wilk test._ENREF_23 Associations were analyzed by a chi square test, Fisher's exact test, Wilcoxon rank-sum test, Spearman's rank-order correlation and linear and logistic regressions.

Sensitivity analysis was done by adjusting for BMI as pairing was only partially accomplished. For parametric tests, a multiple linear regression was used. For categorical and non-parametric tests, calculations were redone with the above tests while either adjusting for BMI or excluding those with a BMI of 35 or over (mean BMI ± SD: control group 26.9 ± 3.7; nGER group 27.6 ± 2.9) when adjustment calculations were not applicable.

4 Results

4.1 Cohort characteristics

The first two papers were based on international cohorts. The first paper included subjects from Iceland and Sweden, the second paper subjects from Iceland, Sweden and Belgium. Papers III and IV were based on an Icelandic cohort.

The BOLD cohort and the ECRHS III nGER subcohort were similar as to age, gender distribution, and prevalence of hypertension and diabetes. BMI was slightly higher in the ECRHS III nGER subcohort. The ECRHS I and II cohort was approximately 20 years younger at baseline as expected, had slightly fewer males, more current smokers, and a lower BMI (Table 2).

Table 2. Demographic factors of the study cohorts. Data for the ECRHS I and II cohort is baseline data. Abbreviations: BOLD, Burden of Obstructive Lung Disease Initiative; ECRHS, European Community Respiratory Health Survey.

	BOLD cohort (n = 1,325)	ECRHS I and II cohort (n = 1,761)	ECRHS III nGER subcohort (n = 90)
Age, years	57 ± 12	34 ± 7	56 ± 7
Male gender	53%	46%	53%
Hypertension	31%		33%
Diabetes	4.8%		3.3%
BMI (kg/m^2)	27.5 ± 4.7	23.6 ± 3.5	28.3 ± 4.6
Smoking:			
- Never	41%	46%	39%
- Former	42%	24%	46%
- Current	16%	31%	16%

4.1.1 Characteristics of nGER groups

The BOLD cohort was divided into the following three groups: “No nGER” (n = 1,040), “Treated GER” (n = 183), and “nGER” (n = 102). No difference was found between these groups regarding age, gender and diabetes. The treated GER group had a higher BMI, and more prevalent hypertension and cigarette smoking compared to those without nGER. The nGER group was comparable to those without nGER in these respects (see Paper I). Hereafter we will focus mostly on the groups “No nGER” and “nGER”.

The ECRHS I and II cohort was divided into the following four groups: “Never nGER” (n = 1,298), “nGER at baseline” (n = 139), “nGER at follow-up” (n = 201) and “Persistent nGER” (n = 123). No difference was found between these groups regarding gender, BMI change on follow-up, smoking or atopy. Those with new or persistent nGER had a higher baseline BMI and were

more often on anti-acid medication, and those with persistent nGER were older (see Paper II). Hereafter we will focus mostly on the groups “Never nGER” and “Persistent nGER”.

The ECRHS III nGER subcohort was divided into two groups: “No nGER” (n = 42) and “With nGER” (n = 48). No difference was found between these groups regarding age, gender, smoking status or incidence of hypertension or diabetes. Those with nGER had higher BMI, although statistically non-significant (see Papers III and IV).

A sensitivity analysis on the nGER definition in the ECRHS III nGER subcohort was performed. The case and control definition was then widened to all participants with nGER or without nGER at follow-up regardless of baseline symptoms, and resulted in a case group of 53 and a control group of 67 participants. Using this categorization the association described below between nGER and the laryngoscopic findings remained significant (p = 0.02), whereas the association between pepsin in EBC became statistically not significant (p = 0.23).

4.2 Symptom analysis

4.2.1 Respiratory symptoms

In the cross-sectional analysis on the BOLD and ECRHS III nGER subcohort, an association was found between nGER and various respiratory symptoms. Specifically, chronic cough and phlegm were more common among nGER subjects in both cohorts (Table 3).

Current asthma and exacerbations of respiratory symptoms were only evaluated in the ECRHS III nGER subcohort. Current asthma was found to be significantly more common in the nGER group [27% vs 7%, p = 0.01], as well as exacerbations of respiratory symptoms [19% vs 5%, p=0.04]. This difference was even clearer when looking at frequent exacerbations (Figure 7). Among those with nGER, higher BMI associated with more exacerbations of respiratory symptoms (Figure 8).

In the BOLD cohort, those with treated GER had less respiratory symptoms than those with symptomatic nGER.

Additionally, the longitudinal analysis of the ECRHS I and II cohort showed an increased new onset of these and other respiratory symptoms among those with persistent nGER as well as new-onset asthma (Table 4), even after adjusting for gender, age, location, follow-up time, smoking history, and BMI (Figure 9). Interaction analysis was done while simultaneously adjusting for location, age, gender, follow-up time, smoking history, baseline BMI and change in BMI. The association between persistent nGER and new respiratory symptoms was stronger among women than men [OR (95% CI): 21.6 (2.8 – 163.2) vs 1.7 (0.8 – 3.6), $p_{interaction}$ = 0.02].

Table 3. Prevalence of respiratory symptoms in two cross-sectional studies on nGER, with similar populations but different nGER definitions and cohort sizes. Abbreviations: BOLD, Burden of Obstructive Lung Disease Initiative; ECRHS, European Community Respiratory Health Survey; nGER, nocturnal gastroesophageal reflux; mo/yr, months per year.

	BOLD cohort			**ECRHS III nGER subcohort**		
	No nGER (n = 1,040)	nGER (n = 102)	p-value	No nGER (n = 42)	Persistent nGER (n = 48)	p-value
Wheeze	21.9	**42.2**	**<0.001**	16.7%	27.1%	0.24
Wheeze and breathlessness	6.1	**22.6**	**<0.001**	4.8%	14.6%	0.12
Wheeze when not having a cold				14.3%	22.9%	0.30
Nocturnal chest tightness				0.0%	**14.6%**	**0.01**
Breathlessness at rest				0.0%	**14.6%**	**0.01**
Breathlessness after exercise	18.8	27.9	0.15	9.5%	**31.3%**	**0.01**
Nocturnal attacks of breathlessness				0.0%	8.3%	0.06
Nocturnal cough				14.3%	29.2%	0.09
Chronic morning cough				9.5%	**29.2%**	**0.02**
Chronic cough				14.3%	**33.3%**	**0.04**
Coughs most days at least 3 mo/yr.	9.3	**15.7**	**0.04**	11.9%	27.7%	0.07
Chronic morning phlegm				19.1%	**39.6%**	**0.03**
Chronic phlegm				9.5%	20.8%	0.14
Phlegm most days at least 3 mo/yr.	9.6	**19.6**	**0.002**	18.0%	**37.8%**	**0.05**

Table 4. New onset of respiratory symptoms among subjects with persistent nGER in a 9 year prospective study. Abbreviations: ECRHS, European Community Respiratory Health Survey; nGER, nocturnal gastroesophageal reflux.

	ECRHS I and II cohort		
	Never nGER (n = 1,298)	Persistent nGER (n = 123)	p-value
Wheeze	11.3	**22.2**	**0.01**
Wheeze and breathlessness	6.0	**16.0**	**<0.001**
Wheeze when not having a cold	7.8	**15.7**	**0.01**
Nocturnal chest tightness	7.5	**17.1**	**0.003**
Breathlessness at rest	3.9	**8.6**	**0.03**
Breathlessness after exercise	9.1	**18.2**	**0.01**
Nocturnal attacks of breathlessness	3.1	**7.4**	**0.03**
Nocturnal cough	19.5	**36.1**	**0.001**
New-onset asthma	5.4	**13.0**	**0.002**

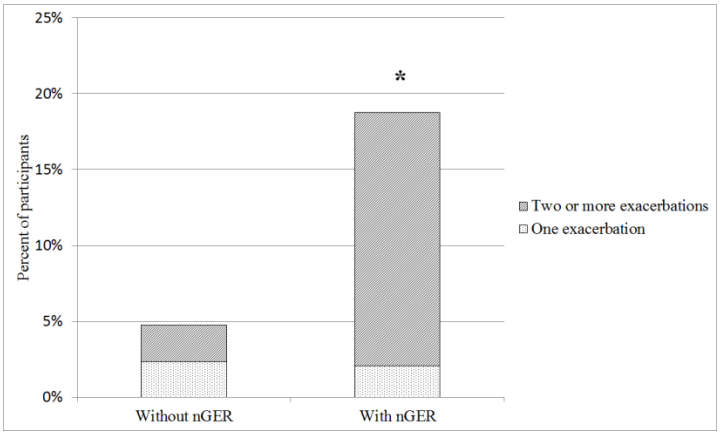


Figure 7. Exacerbations of respiratory symptoms in the previous twelve months among subjects with or without nGER.

* p = 0.02 for frequent exacerbations, by chi square test.

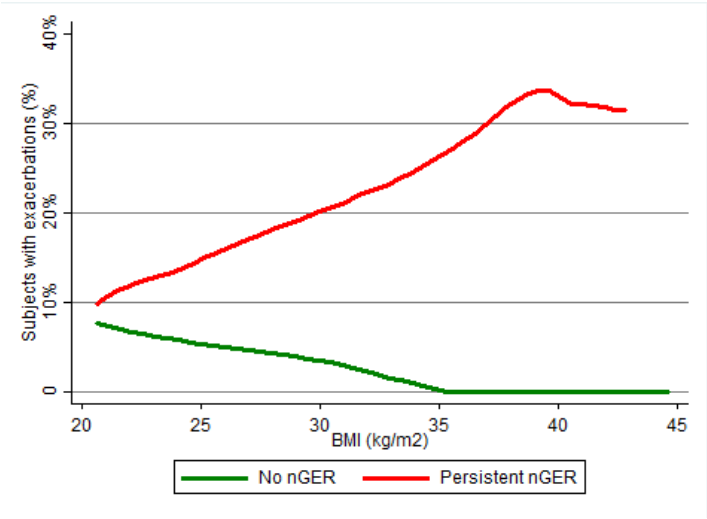


Figure 8. The association between BMI and exacerbations of respiratory symptoms by nGER status. Graph type: Kernel-weighted local polynomial smoothing (kernel bandwidth = 5). P-values by linear regression (with BMI inverted, 1/BMI, to achieve normal distribution): Green line: 0.095, Red line: 0.046.

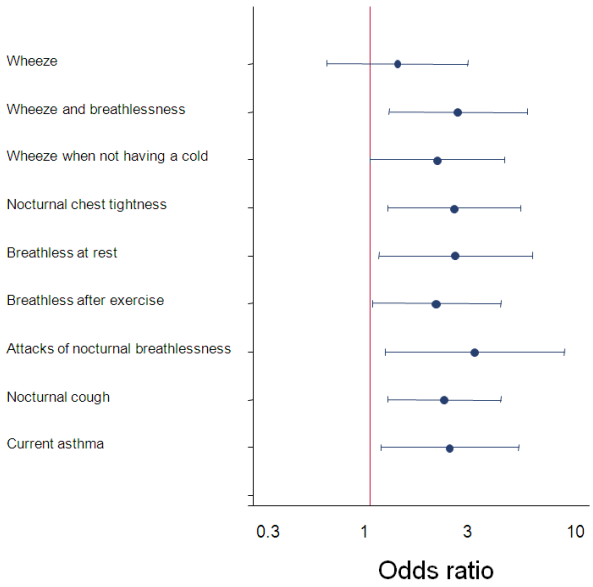


Figure 9. Odds ratios and 95% confidence intervals by logistic regression for the association between new-onset of respiratory symptoms and persistent nocturnal gastroesophageal reflux (nGER) compared to never nGER, adjusted for gender, age, location, smoking history at follow-up, body mass index (BMI) at baseline and change in BMI.

4.2.2 Symptoms of obstructive sleep apnea

In the cross-sectional analysis on the BOLD and ECRHS III nGER subcohort, an association was found between nGER and symptoms of OSA. In the BOLD cohort, observed apneas were significantly more prevalent among those with nGER, but observed snoring was not significantly more prevalent. The contrary was true for the ECRHS III nGER subcohort (Table 6). In both cohorts, daytime sleepiness was more prevalent among nGER subjects as measured by a single question, but the ESS score was not significantly increased (Table 6). Interaction analysis was performed on the BOLD data while adjusting for center, age, gender, smoking history and BMI. The analysis showed that the association between nGER and any OSA symptom was stronger in subjects from Reykjavík than from Uppsala [OR (95% CI): 3.60 (1.86 – 7.00) vs 1.42 (0.81 – 2.49), $p_{interaction}$ = 0.04]. Also, this same association was stronger among women than men [OR (95% CI): 3.55 (1.86 – 6.78) vs 1.42 (0.80 – 2.51), $p_{interaction}$ = 0.04].

In the BOLD cohort, those with treated GER had less OSA symptoms than those with symptomatic nGER.

The longitudinal analysis of the ECRHS I and II cohort showed an increased new onset of observed apneas and more daytime sleepiness measured by the ESS among those with persistent nGER (Table 5), even after adjusting for gender, age, location, follow-up time, smoking history, and BMI.

Table 5. Onset of OSA symptoms among subjects with persistent nGER in a 9 year prospective study. Abbreviations: ECRHS, European Community Respiratory Health Survey; nGER, nocturnal gastroesophageal reflux.

	ECRHS I and II cohort		
	Never nGER (n = 1,298)	Persistent nGER (n = 123)	p-value
Loud snoring ≥ 3 nights a week	19.9	25.6	0.22
Observed apneas ≥ 1 night a week	2.7	**10.6**	**<0.001**
Daytime sleepiness ≥ 3 days a week	26.3	35.3	0.16
Epworth Sleepiness Scale	6.1 ± 3.7	**8.1 ± 4.4**	**<0.001**

Table 6. Prevalence of OSA symptoms in two cross-sectional studies on nGER, with similar populations but different nGER definitions and cohort sizes. Abbreviations: BOLD, Burden of Obstructive Lung Disease Initiative; ECRHS, European Community Respiratory Health Survey; nGER, nocturnal gastroesophageal reflux.

	BOLD cohort			**ECRHS III nGER subcohort**		
	No nGER (n = 1,040)	nGER (n = 102)	p-value	No nGER (n = 42)	Persistent nGER (n = 48)	p-value
Loud snoring ≥ 3 nights a week	22.8	30.7	0.07	23.8%	**44.7%**	**0.04**
Observed apneas ≥ 1 night a week	5.1	**10.8**	**0.02**	2.4%	12.5%	0.08
Daytime sleepiness ≥ 3 days a week	20.5	**42.2**	**<0.001**			
Daytime sleepiness, considerable or more				9.8%	**46.8%**	**<0.001**
Epworth Sleepiness Scale	5.9 ± 3.9	6.9 ± 4.6	0.18	6.6 ± 4.0	8.2 ± 5.4	0.12

4.3 Measurements

4.3.1 Lung function

Lung function data differed between the study populations. Those with nGER in the ECRHS III nGER subcohort had similar post-bronchodilator FEV1, FVC, FEV1/FVC and reversibility compared to controls (Table 7). However, subjects with nGER in the BOLD cohort had a significantly lower FEV1/FVC than subjects without nGER. COPD, as diagnosed by post-bronchodilator spirometry (FEV1/FVC < 0.70 or below LLN), was significantly more common in the group with nGER (Table 7). Additionally, COPD was less common in the treated GER group than in the symptomatic nGER group. These associations remained significant after adjusting for centre, age, gender, smoking history and BMI.

Table 7. Post-bronchodilator lung function data from two cross-sectional studies on nGER, with similar populations but different nGER definitions and cohort sizes. Abbreviations: BOLD, Burden of Obstructive Lung Disease Initiative; ECRHS, European Community Respiratory Health Survey; nGER, nocturnal gastroesophageal reflux.

	BOLD cohort		
	No nGER (n = 1,040)	nGER (n = 102)	p-value
FEV1 (% predicted)	94.8 ± 15.8	92.2 ± 17.5	0.13
FVC (% predicted)	95.5 ± 13.4	95.5 ± 14.6	0.97
FEV1/FVC	0.76 ± 0.08	**0.74 ± 0.10**	**0.02**
FEV1/FVC < 0.70	15.6%	**25.0%**	**0.02**
	ECRHS III nGER subcohort		
	No nGER (n = 42)	Persistent nGER (n = 48)	p-value
FEV1 (% predicted)	97.9 ± 14.5	94.1 ± 15.6	0.24
FVC (% predicted)	96.6 ± 13.8	93.7 ± 13.2	0.33
FEV1/FVC	0.78 ± 0.05	0.78 ± 0.09	0.92
FEV1/FVC < 0.70	2.5%	10.9%	0.13

Interaction analysis was performed on the BOLD data while adjusting for center, age, gender, smoking history and BMI. The analysis showed a significant difference between subjects with a normal BMI (20-25) and obese subjects (>30) in the association between nGER and FEV1. Among normal weight subjects, those with nGER had significantly lower FEV1 than those without nGER, while no significant association of this sort was found among obese subjects [coef. (95% CI): -8.10 (-14.51, -1.70) vs 3.62 (-2.44, 9.67), $p_{interaction}$ = 0.01]. Finally, the association between nGER and lower FEV1/FVC was stronger among men than women [coef. (95% CI): -4.22% (-6.36%, -2.09%) vs -0.14% (-2.45%, 2.17%), $p_{interaction}$ = 0.009].

In the ECRHS I and II cohort, pre-bronchodilator spirometry was performed in 1417 persons and methacholine challenge in 976 persons. No significant associations were found between nGER status and change in FEV1, FVC or FEV1/FVC. New onset of BHR during the study period was significantly higher among subjects with persistent nGER, however [7.0% vs 14.8%, p = 0.04]. This association was not statistically significant after adjusting for age, gender, location, follow-up time, smoking history, BMI at baseline and change in BMI [adjusted OR (95% CI): 2.01 (0.81 - 4.96)].

4.3.2 Laryngoscopy

Altogether 105 subjects from the ECRHS III nGER subcohort underwent laryngoscopy. The average ICC for the RFS between the three raters was 0.48. Those with persistent nGER had a higher RFS compared to controls [Mean ± SD: 5.1 ± 2.3 vs 3.9 ± 2.2, p = 0.02]. A positive laryngoscopy for laryngopharyngeal reflux was more common among those with persistent nGER [7% vs 29%, p = 0.02]. The association between nGER and a higher RFS remained significant after adjusting for smoking and chronic sinusitis (p = 0.03).

4.3.3 Esophageal 24h MII-pH monitoring

An esophageal 24h MII-pH monitoring was done on 16 cases and three control subjects from the ECRHS III nGER subcohort. All three control subjects had a normal 24h MII-pH monitoring. Six out of 16 nGER subjects had confirmed acidic GER (38% vs 0%). On the impedance monitoring, 10 nGER subjects had significant GER (63%). The symptom index was higher among the nGER subjects (0.45 ± 0.38 vs 0 ± 0). Taken together, having a positive 24h MII-pH monitoring (acidic GER, positive impedance monitoring or positive symptom index) was more common among those with persistent nGER than controls (69% vs 0%).

4.3.4 Home sleep studies

In the ECRHS III nGER subcohort, AHI was significantly higher among nGER subjects than controls, as well as audio measured snoring (Table 8). Having both nGER and AHI ≥ 15 was associated with more bronchitis symptoms, whereas having either nGER or AHI ≥ 15 was not. Snoring was positively associated with exacerbations of respiratory symptoms among those with nGER, but not among those without nGER (Figure 10). Among those with nGER, snoring was positively associated with exacerbations only among subjects with measurable pepsin in EBC (Table 9).

After adjusting for BMI, AHI no longer associated with nGER, but measured snoring was similarly associated with nGER after adjusting for BMI. No other significant differences were found after adjusting for BMI.

Table 8. Home sleep study results from the ECRHS III nGER subcohort. Abbreviations: ECRHS, European Community Respiratory Health Survey; nGER, nocturnal gastroesophageal reflux; OSA, obstructive sleep apnea; AHI, apnea-hypopnea index; IQR, interquartile range.

	ECRHS III nGER subcohort		
	No nGER (n = 42)	Persistent nGER (n = 48)	p-value
Moderate/severe OSA (AHI ≥ 15)	10.5%	26.2%	0.07
AHI (median (IQR))	1.9 (0.5 - 8.0)	**4.8 (1.4 - 16.2)**	**0.03**
Snore index (snores/hour of sleep) (median (IQR))	67 (32 – 182)	**177 (79 – 281)**	**0.004**

Table 9. Results from a logistic regression on the association between snoring and prevalence of exacerbations among subjects with persistent nGER, either with positive or negative pepsin in EBC.

	ECRHS III nGER subcohort	
	Odds ratio for exacerbations / 100 snore index	p-value
Persistent nGER, negative pepsin (n = 13)	1.8 (0.5 – 6.3)	0.37
Persistent nGER, positive pepsin (n = 29)	3.8 (1.2 – 11.4)	0.02

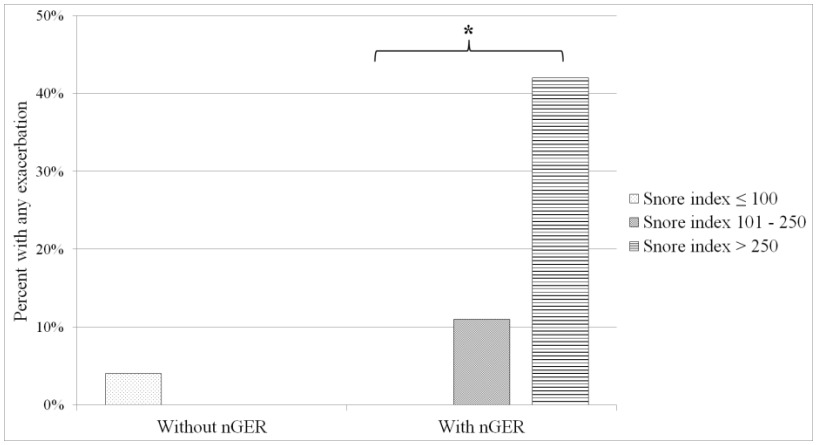


Figure 10. A positive association was found between snoring and exacerbations of respiratory symptoms among subjects with nGER, but not among subjects without nGER.
* p = 0.03 by linear regression.

4.3.5 Biomarker analysis

In the ECRHS III nGER subcohort, subjects with persistent nGER had more often measurable pepsin in EBC than controls [67% vs 45%, respectively, p = 0.04], and actual pepsin levels in EBC were significantly higher (Figure 11). Substance P and 8-isoprostane were also higher in EBC among subjects with

nGER than those without nGER. In PEx, both albumin and SP-A were lower in subjects with nGER than in those without, while the ratio between albumin and SP-A in PEx was similar in both groups. In plasma samples, no difference was found in SP-A, CRP or IL-8 concentrations between nGER subjects and controls (Table 10).

In the nGER group, IL-8 in plasma was significantly higher among those with exacerbations of respiratory symptoms than in those without exacerbations [median (IQR): 0.2 pg/ml (0.2 – 2.3) vs 1.6 pg/ml (1.2 – 3.1), p = 0.03] (Figure 12a). In the nGER group, substance P was higher among those with nocturnal cough than those without nocturnal cough [708 pg/ml (597 – 793) vs 808 pg/ml (732 – 900), p = 0.03] (Figure 12b). No other significant differences were found in biomarkers in the nGER group in relation to exacerbations or respiratory symptoms.

These findings did not change after adjusting for BMI.

Table 10. Biomarker measurements in exhaled air and plasma from the ECRHS III nGER subcohort. Values presented as "median (interquartile range)" unless otherwise stated. Abbreviations: ECRHS, European Community Respiratory Health Survey; nGER, nocturnal gastroesophageal reflux; SP-A, surfactant protein A; IL-8, interleukin 8; FeNO, fraction of exhaled nitric oxide; hs-CRP, high sensitivity C-reactive protein; ppb, parts per billion.

	ECRHS III nGER subcohort		
	No nGER (n = 42)	Persistent nGER (n = 48)	p-value
Exhaled air:			
FeNO (ppb)	15 (13 - 20)	17 (11 - 25)	0.48
EBC:			
Substance P (pg/ml)	623 (562 - 676)	**741 (626 - 821)**	**<0.001**
Neurokinin A (% detected)	17%	30%	0.13
Pepsin (ng/ml)	0.8 (0.8 - 3.6)	**2.5 (0.8 - 5.8)**	**0.03**
IL-8 EBC (% detected)	12%	19%	0.37
8-isoprostane (pg/ml)	2.6 (2.2 - 2.9)	**3.0 (2.7 - 3.9)**	**0.002**
PEx:			
Albumin (mg/g*)	73 (57 - 91)	**48 (31 - 69)**	**<0.001**
SP-A (mg/g*)	38 (31 - 43)	**25 (20 - 35)**	**<0.001**
Albumin/SP-A (ratio)	1.92 (1.50 - 2.64)	1.74 (1.26 - 2.95)	0.79
Plasma:			
hs-CRP (mg/l)	1.05 (0.68 - 2.26)	1.08 (0.62 - 1.94)	0.96
IL-8 plasma (% detected)	57%	52%	0.63
SP-A (ng/ml)	34.7 (29.8 - 48.6)	35.6 (27.6 - 47.0)	0.81

* Measured as mg of protein per gram of exhaled particles.

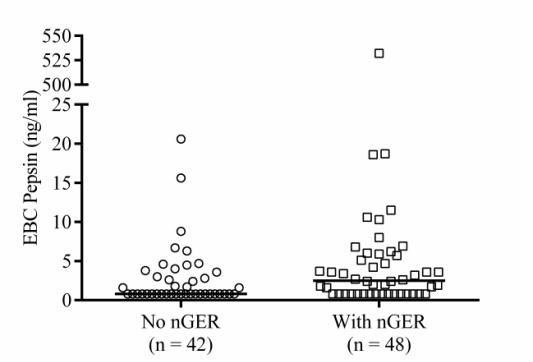


Figure 11. Pepsin levels in exhaled breath condensate samples were significantly higher among subjects with persistent nGER than controls. The transverse line stands for the median value. Samples with undetectable pepsin levels were registered as 0.8 ng/ml (half of the lower detection limit).
p = 0.03, by Wilcoxon rank-sum test.

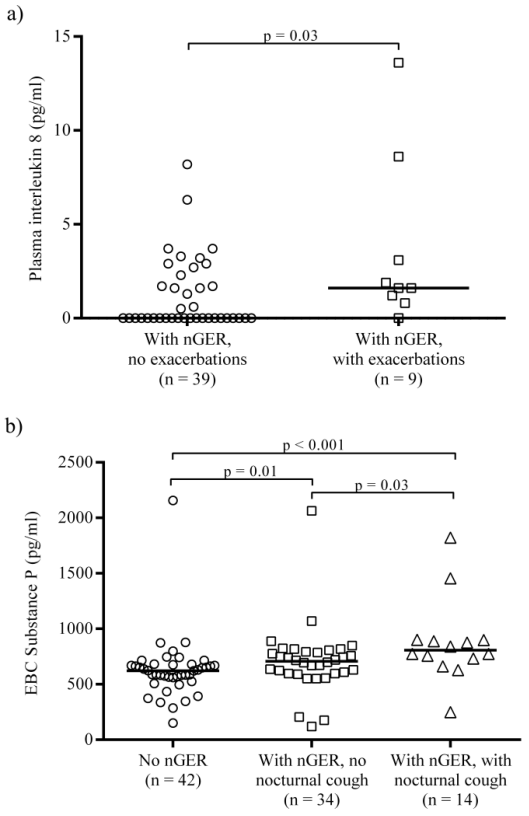


Figure 12. a) Interleukin 8 levels in plasma among nGER subjects with or without exacerbations of respiratory symptoms. The transverse line represents median value. b) Substance P levels in exhaled breath condensate among subjects with nGER with or without nocturnal cough. The transverse line represents median value.
P-values calculated with Wilcoxon rank-sum test.

5 Discussion

In the current studies, nocturnal gastroesophageal reflux was significantly associated with respiratory symptoms, such as asthma and bronchitis symptoms, as well as exacerbations of these symptoms. Having persistent nGER was associated with more frequent onset of respiratory symptoms, indicating that nGER precedes the respiratory symptoms. Symptoms of SDB/OSA were also more common among those with nGER, and persistent nGER associated with more onset of these symptoms. Subjects with persistent nGER had a higher number of measured events on a sleep study, where the strongest association was found with snoring, indicating increased upper airway resistance during sleep. Subjects with persistent nGER had higher pepsin and higher inflammatory biomarkers in exhaled air. The biomarker findings support both theories on micro-aspiration of gastric fluids (increased pepsin and 8-isoprostane in EBC) and neurogenic inflammation through a vagal reflex (increased substance P in EBC) as etiological mechanisms of nGER related respiratory symptoms, but the symptom profile seems to differ between these two mechanisms.

5.1 Prevalence of nGER

The prevalence of nGER in our studies varied based on the definition used. In our general population sample in the BOLD study, aged 40 years and older, the prevalence of nGER symptoms once a week or more was almost 8%. A previous study on young adults in ECRHS I (age range 20-48 years) using the same definition for nGER found the prevalence to be 5% (Gislason et al., 2002). A possible reason for this difference was the 3.5 point higher average BMI in the BOLD cohort compared to the ECRHS I cohort. Another reason might be an increase in nGER prevalence with increasing age. However, the mean age was not different between subjects with or without nGER in either study, and no difference in nGER prevalence was found between age groups in the BOLD cohort (data not shown), thus indicating that age was not a significant factor. Also, a recent systematic review found an increasing GERD prevalence worldwide, possibly independent of age or BMI changes (El-Serag et al., 2014). Irrespective of the cause, nGER seemed to be a growing problem over time in our studies.

The prevalence of persistently symptomatic nGER over a 9 year period in ECRHS I and II, including all frequencies of nGER symptoms, was 7%. With more strict criteria for nGER symptoms once a week or more, the prevalence fell to 2%. These results showed that nGER symptoms vary over time, where only a subgroup of subjects consistently report nGER symptoms. Interestingly, very similar ratios between consistent vs inconsistent nGER symptom groups were observed in the ECRHS III as in the ECRHS I and II study, even though the time to follow-up varied from 6 months to 9 years.

These findings suggest that the variability in symptom prevalence needs to be accounted for in studies on nGER, either by selecting only significantly symptomatic subjects or subjects with persistent symptoms. However, given the available and effective GER treatment, observing symptomatic patients obviously has ethical considerations.

5.2 Validity of nGER definition

Only data from the ECRHS III study allowed us to explore the appropriateness of our nGER definition. In that study, we found a persistent report of nGER symptoms on two occasions to be associated with significant GER on a 24h MII-pH measurement, signs of airway inflammation on laryngoscopy, and reflux of gastric contents to the airways measured by pepsin in exhaled air.

The results from the 24h MII-pH measurement showed that 69% of the 16 measurements on persistent nGER subjects were positive for GER, illustrating that the questionnaire method identifies a group with significant GER. This showed that even though the persistent nGER subjects had relatively mild nocturnal symptoms, many of them nonetheless had a positive 24h MII-pH measurement. Currently, an esophageal 24h MII-pH measurement is considered the gold standard for diagnosing GER disease. However, this measurement carries significant drawbacks, being relatively invasive, expensive and troublesome for the subjects (Wong et al., 2005). It is also somewhat unclear which parameters are most important in extra-esophageal manifested GER (Blondeau et al., 2007; Sifrim et al., 2005). It is even possible that a longer measurement time, 48h instead of 24h, would be preferable in this group (Swidnicka-Siergiejko & Dabrowski, 2013). These measurements are therefore difficult to use in studies on general populations, and among the 8 controls invited in our study for an esophageal 24h MII-pH measurement, only three accepted, much lower than the participation rate for other study parts.

Our definition of persistent nGER in the ECRHS III was also associated with signs of laryngopharyngeal reflux. This supports the conclusion that our symptom-based nGER definition identifies subjects with significant proximal reflux, as laryngopharyngeal reflux indicates that gastric contents have caused local inflammation in the larynx. A study on laryngeal mucosal biopsies from laryngopharyngeal reflux patients showed a high degree of intracellular pepsin, supporting the conclusion that the condition results from gastric contents affecting the upper airways (Jiang et al., 2011).

Thirdly, pepsin in EBC was more commonly detected among those with persistent nGER than among controls. The finding of pepsin in exhaled air is suggestive of gastric contents having reached the airways (Bardhan et al., 2012). Pepsin has been shown to be harmful to airway epithelial cells, especially but not exclusively in an acidic milieu (Bathoorn et al., 2011; Johnston et al., 2012). However, as reflux episodes happen intermittently, often at long intervals, and not all reach the airways, false negative measurements are probably common (Zerbib et al., 2005). Indeed, previous

studies have found a discrepancy between 24h MII-pH measurements and pepsin in bronchoalveolar lavage samples (Rosen et al., 2012). In our data, pepsin was not increased among those reporting nGER symptoms only on one of two visits. This indicated that those with mild, occasional nGER symptoms probably had too little proximal reflux to be subjects for studies on the respiratory effects of GER. Further supporting this decision was our finding from the ECRHS I and II cohort, where persistent nGER was shown to be more strongly associated with new onset of respiratory symptoms than "new-onset" nGER.

Of note, surprisingly many control subjects in our study had measurable pepsin in their exhaled air. This may have been caused by normal, intermittent, physiological reflux episodes in some cases (Zerbib et al., 2005). However, it was unclear if this could explain that as many as 45% of the control subjects had positive measurements. Other possible explanations were that pepsinogen, which is produced to a small degree in alveolar cells, might have contaminated some samples (Elabiad & Zhang, 2011). Also, a cross-reaction may have occurred, although no cross-reactions with other proteins were described with the kit used for pepsin measurements.

5.3 Epidemiology

5.3.1 Respiratory symptoms

In all studies for this dissertation, symptoms of chronic bronchitis and asthma were more common among nGER subjects than among those without nGER. This is in accordance with results from other studies, which show an association of GER with chronic cough, asthma and various upper respiratory tract symptoms (El-Serag & Sonnenberg, 1997; Fontana & Pistolesi, 2003; Gislason et al., 2002; Vakil et al., 2006). Furthermore, we found that persistent nGER was associated with exacerbations of these respiratory symptoms.

In the 9 year follow-up study on 1,761 subjects, subjects with persistent nGER were roughly twice as likely to report new respiratory symptoms at follow-up as those without nGER. This held true even after adjusting for confounding factors. Previous studies have reported associations between GER and chronic cough, asthma and various upper respiratory tract symptoms (Fontana & Pistolesi, 2003; Gislason et al., 2002). Our results add prospective data to these known associations, thereby supporting theories on causality in these associations.

_ENREF_4Our study indicated that nGER was a risk factor for developing asthma, as the new onset of asthma was greater than twofold among those with persistent nGER compared to those without nGER. This did not change after adjusting for confounding factors. On the other hand, a study on the UK General Practice Research Database found evidence for asthma preceding GER (Ruigomez et al., 2005), suggesting the causality might be bidirectional.

Our finding of an association between nGER and exacerbations of respiratory

symptoms, especially among obese subjects, may be of considerable clinical importance. Previous studies have also shown GER to be associated with COPD and asthma exacerbations (Simpson et al., 2014; Terada et al., 2008). These exacerbations cause significant impairment of health, and affect lung function negatively over time (Celli et al., 2008; Jones et al., 2014; Kupczyk et al., 2014). In COPD, the exacerbation frequency is a positive predictive factor for morbidity and mortality (Suissa et al., 2012). It is therefore important to learn more about the possible reasons for exacerbations, both in asthma and COPD, to be able to treat and prevent them more effectively. Our results, together with the biomarker measured in exhaled air, indicate that nGER should be studied further as a potential causative factor. The results of the exhaled biomarkers in relation to nGER and exacerbations is further discussed in section 5.5.

5.3.1.1 Antireflux treatment

With reservation for the cross-sectional nature of the BOLD study, those with treated GER had fewer respiratory symptoms than those with symptomatic nGER. This indicated that GER medication may have been beneficial against these symptoms, although a randomized controlled study would have better served to answer that question. In the longitudinal ECRHS I and II study, we did not find a significant effect of nGER treatment on asthma outcomes. The reason for this discrepant association with nGER treatment may be due to the fact that our data collection on nGER treatment did not differ between the type of medication used, the dosage or total treatment time. Previous studies on GER treatment in asthma patients have shown inconsistent results (Coughlan et al., 2001; Fontana & Pistolesi, 2003; Kiljander et al., 2000). A high quality study by Kiljander *et al*. observed that Esomeprazole 40 mg twice daily during 26 weeks induced minor improvements in FEV1 and asthma-related quality of life symptoms in asthmatic patients with GER, compared to placebo treatment (Kiljander *et al.*, 2010). The improvement was considered to be of small clinical significance. Other studies with different doses and time spans have given variable results. In a systematic review on medical GER treatment in adult asthmatics, only studies with a long treatment time showed a modest treatment benefit in terms of asthma outcomes (Coughlan et al., 2001; Gibson et al., 2003; Holbrook et al., 2012). A small double-blind study on the effect of pantoprazole therapy for laryngopharyngeal reflux did not find an effect of pantoprazole on laryngeal symptoms (Wo et al., 2006). However, symptoms worsened post treatment in the pantoprazole group, indicating a possible acid-rebound effect.

Interestingly, surgical interventions for GER, which ameliorate the reflux itself, have on the other hand shown a clear improvement on asthma and chronic cough, even after a few years of follow-up (Hu et al., 2015; Liang et al., 2015; Lugaresi et al., 2015). We therefore hypothesize that the modest effects of GER treatment on asthma might rather be explained by an inappropriate treatment target when treating with medicine. Indeed, PPIs do not inhibit the reflux itself but only make the gastric contents less acidic (Clayton et al., 2012; Hemmink et al., 2008) and can therefore only limit but not eliminate the

potential damage of gastric contents reaching the airways through reflux. When all of the above is taken into consideration, it must be considered plausible that nGER may be implicated in the pathogenesis of respiratory symptoms in a subgroup of patients, but better treatment is needed.

5.3.2 OSA symptoms

Overall, the current studies revealed a positive association between nGER and OSA symptoms, such as observed apneas, snoring and daytime sleepiness. The results varied slightly between studies, where observed apneas were arguably most associated with nGER, followed by daytime sleepiness.

Studying the association between OSA and GER poses certain problems. As the two conditions are rather common, and both have obesity as a main risk factor, the methodology needs to take these two aspects into account (Basoglu et al., 2015; Dent et al., 2005). It is also unclear which comes first, whether OSA leads to GER or vice versa. The results from our longitudinal study on the ECRHS I and II cohort showed that those with persistent nGER more commonly developed symptoms of OSA than those without nGER. This association was independent of BMI. Another study found an association between the laryngeal inflammation in laryngopharyngeal reflux and OSA severity (Payne et al., 2006). As snoring and apneas are caused by upper airway narrowing and recurrent, intermittent upper airway collapse (Owens et al., 2008), the laryngeal edema may predispose subjects for obstructive episodes. Treating subjects with mild OSA and GER with PPIs has in one study shown measurable improvement in both laryngeal inflammation and sleep quality, even though the AHI was not affected (Orr et al., 2009). A recent meta-analysis on six small studies on the effects of PPI treatment on OSA found indications of improved sleep quality without any effect on the AHI (Rassameehiran et al., 2016).

On the other hand, OSA may also induce nGER. As mentioned earlier, nGER may be caused by “fatigue” in the lower esophageal sphincter among untreated OSA subjects (Kuribayashi et al., 2010). Additionally, treating OSA with continuous positive airway pressure has been shown to effectively diminish nGER symptoms, as well as increase pressure in the lower esophageal sphincter (Green et al., 2003; Shepherd et al., 2007). The available data therefore support the conclusion that the relationship between nGER and OSA might be bidirectional.

Daytime sleepiness may be a specific disease entity among GER patients. Numerous studies have found GER subjects to have increased daytime sleepiness and poorer sleep quality (Gerson & Fass, 2009; Gislason et al., 2002; Guda et al., 2004; Kerr et al., 1992). One study found that the grade of esophagitis among GER subjects correlated positively with the degree of daytime sleepiness (Demeter et al., 2004). PPI treatment may even improve daytime sleepiness among these subjects (Orr et al., 2009). Therefore, GER needs to be evaluated further as a differential diagnosis for patients with daytime sleepiness with or without OSA, preferably with a validated

questionnaire.

One notable finding from our BOLD cohort which we have not seen reported before was the proportionally stronger association between OSA symptoms and nGER among women than men, even after the usual adjustments. We did not find an explanation for this gender difference.

Interestingly, objectively measured snoring had a stronger association with nGER than subjectively reported snoring. The sleep studies performed on the ECRHS III nGER subcohort showed the strongest association was between nGER and audio measured snoring. In contrast to the association with AHI, the association with audio measured snoring was significant even after adjusting for BMI. This association with snoring has been reported previously, but is much less studied than the association with AHI (Charaklias et al., 2013). A study on subjects undergoing polysomnography demonstrated that all self-reported snorers had a significantly higher prevalence of GER than the general population, independent of AHI (Basoglu et al., 2015). It is even possible that the increased respiratory effort associated with snoring weakens the lower esophageal sphincter even more than the traditionally measured apneas. Further studies are needed on nGER and objectively measured snoring, which ideally should be done by audio measurements (Arnardottir et al., 2015b).

5.3.3 Association between sleep-disordered breathing, nGER and respiratory symptoms

Both nGER and snoring had a synergistic effect on the prevalence of exacerbations of respiratory symptoms. Previous studies have shown such exacerbations to be associated with nGER, as well as SDB (Alkhalil et al., 2009; Terada et al., 2008), but to our knowledge this synergistic effect has not been described before. As this effect was more pronounced among those with measurable pepsin in EBC, we hypothesize that SDB-associated nGER causes exacerbations of respiratory symptoms by micro-aspirations of gastric contents.

5.4 Lung function

The lung function data differed between study cohorts. Only in the BOLD cohort did we find significant associations between key lung function parameters and nGER status. In the BOLD study, nGER associated with COPD as defined by lung function data. Among normal weight subjects, FEV1 was significantly lower among nGER subjects than controls. It is unlikely that age affected the difference between cohorts, as subjects in the BOLD cohort and the ECRHS III nGER subcohort were of similar age. Our results are therefore inconclusive. Other studies have shown variable though often positive results. A study on COPD subjects found GER to be associated with significantly more symptoms and poorer quality of life, but only a marginally lower FEV1 presented as percent predicted (Martinez et al., 2014). A study on US military veterans with reflux esophagitis found COPD, as well as many other respiratory diseases, to be more common than in a

control group (El-Serag & Sonnenberg, 1997). Among 32 GER subjects with esophagitis, a significant decrease was found in FEV1 and PEF, as well as a lower pH and higher lactate dehydrogenase in bronchoalveolar aspirates, indicative of local tissue injury (Mise et al., 2010). One study comparing subjects with erosive GER disease to subjects with non-erosive GER disease found the former to have worse pulmonary function than the latter (Maher & Darwish, 2010). The difference in lung function results may therefore reflect different GER subgroups, where those with erosive GER disease may be more susceptible to respiratory injury with consequent effects on lung function. Further studies are needed to evaluate this hypothesis.

5.5 Biomarkers in exhaled air

The current study on the ECRHS III nGER subcohort revealed an association between persistent nGER, pepsin, 8-isoprostane and substance P in EBC, as well as surfactant protein A and albumin in PEx.

IL-8 in plasma was elevated among participants with both persistent nGER and exacerbations of respiratory symptoms. In support of this, previous studies have found IL-8 to be increased in bronchoalveolar lavage (BAL) from asthmatic children, but only among those with GER (Sacco et al., 2006). Additionally, a correlation between IL-8 and bile acids in BAL has been found in lung transplanted patients who developed bronchiolitis obliterans syndrome (D'Ovidio et al., 2005). We hypothesize that this increase in IL-8 reflects airway inflammation related to GER, suggesting a specific role of IL-8 in neutrophil recruitment in GER related respiratory conditions.

8-isoprostane, a biomarker for oxidative stress, was increased in EBC among those with nGER. This association has previously been described among subjects with asthma, where a positive association was found between GER and 8-isoprostane (Carpagnano et al., 2006). In addition, treating asthma patients with PPI has been shown to reduce 8-isoprostane in EBC only among those with concomitant GER (Shimizu et al., 2007). We therefore propose that elevated 8-isoprostane in EBC among subjects with persistent nGER may reflect GER-related oxidative stress and inflammation of the airways. As gastric contents have shown to provoke similar inflammation by direct contact with airway epithelium *in vitro*, with elevated IL-6 (Bathoorn et al., 2011), we hypothesize that aspiration of gastric contents is a plausible causative factor of elevated 8-isoprostane in EBC.

Significantly higher levels of the neuroinflammatory marker substance P in EBC were observed among those with nGER, especially among those with nocturnal cough, suggestive of a neural mediated association between nGER and nocturnal cough. A previous study on neuroinflammatory markers in GER and chronic cough showed a similar association (Patterson et al., 2007). Distal esophageal acid perfusion has also been shown to stimulate the vagal nerve, with resulting bronchoconstriction and heart rate variation (Amarasiri et al., 2013). We hypothesize that GER-induced vagal stimulation might lead to neurogenic inflammation in the lungs, where cough is the main resulting symptom. This is in accordance with the previously discussed reflex

theory.

Albumin and SP-A levels were lower in PEx among persistent nGER subjects in our study, indicating an effect on the small airways. In a study on lung transplant patients, GER was associated with decreased levels of SP-A in BAL, as well as increased risk of bronchiolitis obliterans syndrome (D'Ovidio et al., 2006). In asthmatics, SP-A in BAL has been found to decrease after allergen exposure, and to be decreased in idiopathic pulmonary fibrosis and among smokers, although contradictory results have been presented (Erpenbeck et al., 2006; Ledford et al., 2014; Phelps et al., 2004). COPD patients have also been shown to have decreased albumin in BAL (Bernard et al., 1992). However, studies also show SP-A in BAL to be increased in sarcoidosis and mild asthma patients, and albumin is often elevated in BAL in inflammatory diseases (Haslam & Baughman, 1999; Ledford et al., 2014). These discrepancies may partly reflect the difficulties in adjusting for the dilution factor in BAL. In contrast, the PExA^{TM} method collects undiluted samples from the lining fluid in the small airways. The reduced proportion of surfactant protein A in the small airways lining fluid may have had an impact on surfactant function and host defense (Goto et al., 2014). In turn, this might in part explain the association between nGER and exacerbations of respiratory symptoms. Together with the findings from the EBC samples, these findings suggested that persistent nGER affects both the proximal and distal airways.

Interestingly, plasma CRP was not associated with nGER. This suggested that the elevated inflammatory markers seen in EBC and PEx represented a local effect of nGER without a systemic effect.

5.6 Strengths and limitations

The key strengths of the studies were the well-defined study populations, relatively large cohorts except for the ECRHS III nGER subcohort, very acceptable response rates, and spirometries performed in a standardized manner by specially trained professionals. A special strength of the ECRHS I and II study was its prospective nature. Additionally, the ECRHS III nGER subcohort was studied with a holistic approach to the many aspects of nGER with respiratory effects, both objectively and subjectively. This included the use of high quality home sleep studies and thorough testing of exhaled biomarkers by several non-invasive methods. As our study populations were based on a general population, the number of severely symptomatic cases was consequently low. This likely resulted in a lower statistical power, but also made our results applicable to the general population._ENREF_46 These studies also had some limitations.

One of the main limitations in this dissertation was the definition of nGER. In the BOLD and ECHRS I and II cohorts, the definition was based on a single question on nocturnal heartburn. This definition can not be seen as diagnostic for GERD (Johnsson et al., 1998; Lacy et al., 2010). To minimize false positives, we confined ourselves in the BOLD cohort to those reporting nocturnal heartburn at least once a week, which is considered a reasonably

specific indicator of GERD (Dent et al., 2005; Klauser et al., 1990). In the ECRHS I and II cohorts, we widened the definition to include all subjects reporting nGER symptoms the previous 4 weeks, but focused mostly on those with persistent symptoms. This resulted in an nGER group of similar size as in the BOLD cohort, roughly indicating that the two definitions identified similarly affected groups. We also ran all the calculations again with nGER defined as heartburn at least once a week or more. This gave very similar results to those with the wider symptom definition, although sometimes not reaching the same statistical significance, as the nGER group included only 34 subjects (data not shown). We therefore believe that the wide definition of nGER used in this study probably did not confound the results.

In the ECRHS III nGER subcohort, the definition was somewhat stronger, based on a validated questionnaire modified to identify subjects with nocturnal symptoms. We did not perform psychometric analyses of our modified RDQ. However, this had previously been done for the well validated and widely used RDQ (Shaw et al., 2001; Vakil et al., 2013). The only change in our version was posing the same questions specifically for daytime and nocturnal symptoms, so new psychometric analyses were considered unnecessary. Another limitation to our case definition was the limited number of 24h MII-pH measurements performed to confirm case selection, which should ideally have been performed on all subjects. However, the invasive nature of these measurements made it difficult to recruit asymptomatic controls and we were only able to recruit three control subjects. From the few measurements done, we found a significant proportion of our nGER group had a positive 24h MII-pH monitoring.

All studies had a certain risk of translation bias. However, we consider this risk to have been small, as the questions were all checked via back-translation and tested for translation bias. When studying the associations of a single entity to many variables, as in this dissertation, the risk of a type I error must be considered. However, as there was a consistent pattern in the many positive results, the risk of a type I error was considered small.

Evaluation of the laryngoscopies in the ECRHS III nGER subcohort showed a significant inter-rater variability. Intraclass correlation for the reflux finding score showed only a moderate reliability for the average score, which was lower than expected even though sufficient for its use in the current study. Previous studies have shown a variable agreement between raters of laryngoscopies, from poor to excellent (Belafsky et al., 2001; Branski et al., 2002; Payne et al., 2006). This might be caused by methodological differences, as in our study the scorers were not previously trained in using the RFS and had not coordinated their scoring methods. In addition, the subjects in our general population cohort had less variation in their scores, which probably magnified the inter-rater variability. Also, a few of the videos were of suboptimal quality.

Our definition of GER treatment in the BOLD cohort did not differentiate between regular or on demand usage, or between different types of

medications. Therefore, even subjects on minimal treatment were categorized as having GER treatment, causing individuals with minimal disease to be classified as treated GER. However, this also served to purify the group without nGER, making it a better reference group.

The analysis of OSA symptoms in the BOLD and ECHRS I and II cohorts was based on subjective measurements only, which are only fairly sensitive to an OSA diagnosis (Maislin et al., 1995; Yuceege et al., 2015). This became evident in the ECRHS III nGER subcohort, where some discrepancy was found between sleep study results and reported apneas and snoring. However, there was still a fair agreement between the objective and subjective scorings, indicating that the subjective symptom reports were usable for assessing snoring and apneas.

In the ECRHS III nGER subcohort, biosamples were collected at different time points during the day, and therefore the biomarker measurements could be affected by circadian variability. However, as participants' visits were similarly spread over time of day, circadian variability was unlikely to affect our results.

There is some uncertainty regarding where the biomarkers measured in EBC are derived from, and the water-vapour dilution of these samples has been shown to differ significantly (Esther et al., 2009). Additionally, many of the biomarkers we measured in EBC were near the lower limit of detection, making the measurements somewhat unreliable. However, the biomarkers which were well above the detection limit in EBC, such as pepsin, substance P and 8-isoprostane, differed clearly between the nGER and control group.

6 Conclusions

We found that nGER was associated with respiratory symptoms and OSA, and that persistent nGER was associated with an increased onset of these symptoms at follow-up. Having consistent nGER symptoms over time was associated with increased airway inflammatory biomarkers, indicating that persistency of nGER symptoms was a risk factor for health impairment. However, we did not find convincing evidence for an association between nGER and declining lung function in the general population.

In our well-defined ECRHS III nGER subcohort, we found in addition to the symptom associations above that nGER was associated with exacerbations of respiratory symptoms. These findings were further supported by significant changes in airway inflammatory biomarkers, both in large and small airways. Those with nGER and nocturnal cough had increased signs of neurogenic inflammation. The subgroup of OSA patients with nGER was of special interest, as we found them to have increased bronchitis symptoms. Snoring was also associated with nGER, and when combined with nGER it strengthened the association of nGER with exacerbations of respiratory symptoms.

6.1 Future perspectives

There is still much research to be done on the effects of GER or nGER on the respiratory system. The task is complex, and first of all we need to understand better the pathogenic mechanisms. Until then we cannot reach a gold standard definition on how to diagnose and phenotype these patients. The possible mechanisms of neurogenic inflammation, micro-aspiration and sleep-disordered breathing seem to play specific roles and need to be studied further. Finding reliable methods that allow us to identify those at risk and treat them appropriately based on their phenotype has a public health aspect of great clinical significance.

Much data suggests that reflux-associated chronic cough is related to a central neuronal sensitization process. These patients do often have only a physiological reflux on a 24h MII-pH monitoring but a clear temporal relationship between reflux and cough episodes, and histologically they have dilated intercellular spaces in their esophagus (Borrelli et al., 2014; Smith et al., 2010). Our finding of elevated neuroinflammatory biomarkers in the exhaled air of subjects with nGER and nocturnal cough further strengthens this theory. Future studies need to verify that the gastric contents induce a neural reflex when reaching the distal esophagus, and consequently what specifically in the gastric contents induces this reflex. Only then can we better understand how to identify and treat these patients.

Additionally, much data suggests that GERD-related rejection of lung transplants is caused by aspirations of gastric contents (D'Ovidio et al., 2005; Mertens et al., 2011). Future studies should evaluate whether GERD can be diminished before transplantation, and whether that would then lead to a significant reduction in the number of rejections. Furthermore, we need to study how to identify subjects with chronic aspirations with minimal invasiveness in order to be applicable to a larger population. This may possibly be achieved with measurement of exhaled biomarkers.

Future research on the subgroup of sleep-disordered breathing patients with nGER and respiratory symptoms should focus on clarifying the temporal relationship and identifying the responsible pathogenic mechanism. Of special interest is to study how CPAP treatment affects this relationship.

In summary, identifying patients where respiratory symptoms are caused by nGER is of utmost importance. It would be optimal to have a validated questionnaire-based method to identify the patients at risk, with the support of simple non-invasive tests. That would facilitate a more personalized approach and treatment of this group.

References

[Author not listed]. (1843). On a Peculiar Morbid Affection of the Stomach: Characterised by Regurgitation of Its Contents, without Nausea. *Prov Med J Retrosp Med Sci, 6*(150), 409-413.

Adachi, K., Fujishiro, H., Katsube, T., Yuki, M., Ono, M., Kawamura, A., Rumi, M. A., Watanabe, M., & Kinoshita, Y. (2001). Predominant nocturnal acid reflux in patients with Los Angeles grade C and D reflux esophagitis. *J Gastroenterol Hepatol, 16*(11), 1191-1196.

Alkhalil, M., Schulman, E., & Getsy, J. (2009). Obstructive sleep apnea syndrome and asthma: What are the links? *J Clin Sleep Med, 5*(1), 71-78.

Allen, C. J., & Newhouse, M. T. (1984). Gastroesophageal reflux and chronic respiratory disease. *Am Rev Respir Dis, 129*(4), 645-647.

Almstrand, A. C., Bake, B., Ljungstrom, E., Larsson, P., Bredberg, A., Mirgorodskaya, E., & Olin, A. C. (2010). Effect of airway opening on production of exhaled particles. *J Appl Physiol, 108*(3), 584-588.

Amarasiri, D. L., Pathmeswaran, A., de Silva, H. J., & Ranasinha, C. D. (2013). Response of the airways and autonomic nervous system to acid perfusion of the esophagus in patients with asthma: A laboratory study. *BMC Pulm Med, 13*, 33.

Arnardottir, E. S., Bjornsdottir, E., Olafsdottir, K. A., Benediktsdottir, B., & Gislason, T. (2015a). Obstructive sleep apnoea in the general population: Highly prevalent but minimal symptoms. *Eur Respir J*.

Arnardottir, E. S., Isleifsson, B., Agustsson, J. S., Sigurdsson, G. A., Sigurgunnarsdottir, M. O., Sigurdarson, G. T., Saevarsson, G., Sveinbjarnarson, A. T., Hoskuldsson, S., & Gislason, T. (2015b). How to measure snoring? A comparison of the microphone, cannula and piezoelectric sensor. *J Sleep Res*.

Bardhan, K. D., Strugala, V., & Dettmar, P. W. (2012). Reflux

revisited: Advancing the role of pepsin. *Int J Otolaryngol, 2012*, 646901.

Basoglu, O. K., Vardar, R., Tasbakan, M. S., Ucar, Z. Z., Ayik, S., Kose, T., & Bor, S. (2015). Obstructive sleep apnea syndrome and gastroesophageal reflux disease: The importance of obesity and gender. *Sleep Breath, 19*(2), 585-592.

Bathoorn, E., Daly, P., Gaiser, B., Sternad, K., Poland, C., Macnee, W., & Drost, E. M. (2011). Cytotoxicity and induction of inflammation by pepsin in Acid in bronchial epithelial cells. *Int J Inflam, 2011*, 569416.

Baumeler, L., Papakonstantinou, E., Milenkovic, B., Lacoma, A., Louis, R., Aerts, J. G., Welte, T., Kostikas, K., Blasi, F., Boersma, W., Torres, A., Rohde, G. G., Boeck, L., Rakic, J., Scherr, A., Tamm, M., & Stolz, D. (2016). Therapy with proton-pump inhibitors for gastroesophageal reflux disease does not reduce the risk for severe exacerbations in COPD. *Respirology*.

Belafsky, P. C., Postma, G. N., & Koufman, J. A. (2001). The validity and reliability of the reflux finding score (RFS). *Laryngoscope, 111*(8), 1313-1317.

Benediktsdottir, B., Janson, C., Lindberg, E., Arnardottir, E. S., Olafsson, I., Cook, E., Thorarinsdottir, E. H., & Gislason, T. (2010). Prevalence of restless legs syndrome among adults in Iceland and Sweden: Lung function, comorbidity, ferritin, biomarkers and quality of life. *Sleep Med, 11*(10), 1043-1048.

Berg, S., Hoffstein, V., & Gislason, T. (2004). Acidification of distal esophagus and sleep-related breathing disturbances. *Chest, 125*(6), 2101-2106.

Bernard, A., Marchandise, F. X., Depelchin, S., Lauwerys, R., & Sibille, Y. (1992). Clara cell protein in serum and bronchoalveolar lavage. *Eur Respir J, 5*(10), 1231-1238.

Blondeau, K., Dupont, L. J., Mertens, V., Tack, J., & Sifrim, D. (2007). Improved diagnosis of gastro-oesophageal reflux in patients with unexplained chronic cough. *Aliment Pharmacol Ther, 25*(6), 723-732.

Blondeau, K., Mertens, V., Vanaudenaerde, B. A., Verleden, G. M.,

Van Raemdonck, D. E., Sifrim, D., & Dupont, L. J. (2008). Gastro-oesophageal reflux and gastric aspiration in lung transplant patients with or without chronic rejection. *Eur Respir J, 31*(4), 707-713.

Borrelli, O., Mancini, V., Thapar, N., Ribolsi, M., Emerenziani, S., de'Angelis, G., Bizzarri, B., Lindley, K. J., & Cicala, M. (2014). Dilated intercellular space diameter as marker of reflux-related mucosal injury in children with chronic cough and gastro-oesophageal reflux disease. *Aliment Pharmacol Ther, 39*(7), 733-742.

Branski, R. C., Bhattacharyya, N., & Shapiro, J. (2002). The reliability of the assessment of endoscopic laryngeal findings associated with laryngopharyngeal reflux disease. *Laryngoscope, 112*(6), 1019-1024.

Bredberg, A., Gobom, J., Almstrand, A. C., Larsson, P., Blennow, K., Olin, A. C., & Mirgorodskaya, E. (2012). Exhaled endogenous particles contain lung proteins. *Clin Chem, 58*(2), 431-440.

Buist, A. S., McBurnie, M. A., Vollmer, W. M., Gillespie, S., Burney, P., Mannino, D. M., Menezes, A. M., Sullivan, S. D., Lee, T. A., Weiss, K. B., Jensen, R. L., Marks, G. B., Gulsvik, A., & Nizankowska-Mogilnicka, E. (2007). International variation in the prevalence of COPD (the BOLD Study): A population-based prevalence study. *Lancet, 370*(9589), 741-750.

Burney, P. G., Luczynska, C., Chinn, S., & Jarvis, D. (1994). The European Community Respiratory Health Survey. *Eur Respir J, 7*(5), 954-960.

Busch, R., Han, M. K., Bowler, R. P., Dransfield, M. T., Wells, J. M., Regan, E. A., & Hersh, C. P. (2016). Risk factors for COPD exacerbations in inhaled medication users: The COPDGene study biannual longitudinal follow-up prospective cohort. *BMC Pulm Med, 16*(1), 28.

Campagnolo, A. M., Priston, J., Thoen, R. H., Medeiros, T., & Assuncao, A. R. (2014). Laryngopharyngeal reflux: Diagnosis, treatment, and latest research. *Int Arch Otorhinolaryngol, 18*(2), 184-191.

Campos, G. M., Peters, J. H., DeMeester, T. R., Oberg, S., Crookes, P. F., & Mason, R. J. (1999). The pattern of esophageal acid

exposure in gastroesophageal reflux disease influences the severity of the disease. *Arch Surg, 134*(8), 882-887; discussion 887-888.

Carpagnano, G. E., Resta, O., Ventura, M. T., Amoruso, A. C., Di Gioia, G., Giliberti, T., Refolo, L., & Foschino-Barbaro, M. P. (2006). Airway inflammation in subjects with gastro-oesophageal reflux and gastro-oesophageal reflux-related asthma. *J Intern Med, 259*(3), 323-331.

Carsin, A. E., Zock, J. P., Jarvis, D., Basagana, X., Heinrich, J., Toren, K., Janson, C., Anto, J. M., & Sunyer, J. (2013). Serum total immunoglobulin E is a surrogate of atopy in adult-onset asthma: A longitudinal study. *Int Arch Allergy Immunol, 160*(4), 387-392.

Celli, B. R., Thomas, N. E., Anderson, J. A., Ferguson, G. T., Jenkins, C. R., Jones, P. W., Vestbo, J., Knobil, K., Yates, J. C., & Calverley, P. M. (2008). Effect of pharmacotherapy on rate of decline of lung function in chronic obstructive pulmonary disease: Results from the TORCH study. *Am J Respir Crit Care Med, 178*(4), 332-338.

Charaklias, N., Mamais, C., Pothula, V., & Kumar, B. N. (2013). Laryngopharyngeal reflux and primary snoring: A pilot case-control study. *B-ENT, 9*(2), 89-93.

Chinn, S., Jarvis, D., Luczynska, C. M., Ackermann-Liebrich, U., Anto, J. M., Cerveri, I., de Marco, R., Gislason, T., Heinrich, J., Janson, C., Kunzli, N., Leynaert, B., Neukirch, F., Schouten, J. P., Sunyer, J., Svanes, C., Wjst, M., & Burney, P. G. (2005a). An increase in bronchial responsiveness is associated with continuing or restarting smoking. *Am J Respir Crit Care Med, 172*(8), 956-961.

Chinn, S., Jarvis, D., Melotti, R., Luczynska, C., Ackermann-Liebrich, U., Anto, J. M., Cerveri, I., de Marco, R., Gislason, T., Heinrich, J., Janson, C., Kunzli, N., Leynaert, B., Neukirch, F., Schouten, J., Sunyer, J., Svanes, C., Vermeire, P., Wjst, M., & Burney, P. (2005b). Smoking cessation, lung function, and weight gain: A follow-up study. *Lancet, 365*(9471), 1629-1635; discussion 1600-1621.

Chung, K. F., Wenzel, S. E., Brozek, J. L., Bush, A., Castro, M., Sterk,

P. J., Adcock, I. M., Bateman, E. D., Bel, E. H., Bleecker, E. R., Boulet, L. P., Brightling, C., Chanez, P., Dahlen, S. E., Djukanovic, R., Frey, U., Gaga, M., Gibson, P., Hamid, Q., Jajour, N. N., Mauad, T., Sorkness, R. L., & Teague, W. G. (2014). International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J, 43*(2), 343-373.

Clayton, S. B., Rife, C. C., Singh, E. R., Kalbfleisch, J. H., & Castell, D. O. (2012). Twice-daily proton pump inhibitor therapy does not decrease the frequency of reflux episodes during nocturnal recumbency in patients with refractory GERD: analysis of 200 patients using multichannel intraluminal impedance-pH testing. *Dis Esophagus, 25*(8), 682-686.

Committee, E. I. S. (2002). The European Community Respiratory Health Survey II. *Eur Respir J, 20*(5), 1071-1079.

Conchillo, J. M., Selimah, M., Bredenoord, A. J., Samsom, M., & Smout, A. J. (2007). Air swallowing, belching, acid and non-acid reflux in patients with functional dyspepsia. *Aliment Pharmacol Ther, 25*(8), 965-971.

Corwin, R. W., & Irwin, R. S. (1985). The lipid-laden alveolar macrophage as a marker of aspiration in parenchymal lung disease. *Am Rev Respir Dis, 132*(3), 576-581.

Coughlan, J. L., Gibson, P. G., & Henry, R. L. (2001). Medical treatment for reflux oesophagitis does not consistently improve asthma control: A systematic review. *Thorax, 56*(3), 198-204.

D'Ovidio, F., Mura, M., Ridsdale, R., Takahashi, H., Waddell, T. K., Hutcheon, M., Hadjiliadis, D., Singer, L. G., Pierre, A., Chaparro, C., Gutierrez, C., Miller, L., Darling, G., Liu, M., Post, M., & Keshavjee, S. (2006). The effect of reflux and bile acid aspiration on the lung allograft and its surfactant and innate immunity molecules SP-A and SP-D. *Am J Transplant, 6*(8), 1930-1938.

D'Ovidio, F., Mura, M., Tsang, M., Waddell, T. K., Hutcheon, M. A., Singer, L. G., Hadjiliadis, D., Chaparro, C., Gutierrez, C., Pierre, A., Darling, G., Liu, M., & Keshavjee, S. (2005). Bile acid aspiration and the development of bronchiolitis obliterans after lung transplantation. *J Thorac Cardiovasc Surg, 129*(5),

1144-1152.

Davis, C. S., Gagermeier, J., Dilling, D., Alex, C., Lowery, E., Kovacs, E. J., Love, R. B., & Fisichella, P. M. (2010). A review of the potential applications and controversies of non-invasive testing for biomarkers of aspiration in the lung transplant population. *Clin Transplant, 24*(3), E54-61.

de Bortoli, N., Nacci, A., Savarino, E., Martinucci, I., Bellini, M., Fattori, B., Ceccarelli, L., Costa, F., Mumolo, M. G., Ricchiuti, A., Savarino, V., Berrettini, S., & Marchi, S. (2012). How many cases of laryngopharyngeal reflux suspected by laryngoscopy are gastroesophageal reflux disease-related? *World J Gastroenterol, 18*(32), 4363-4370.

Decalmer, S., Stovold, R., Houghton, L. A., Pearson, J., Ward, C., Kelsall, A., Jones, H., McGuinness, K., Woodcock, A., & Smith, J. A. (2012). Chronic Cough: Relationship between Micro-Aspiration, Gastroesophageal Reflux and Cough Frequency. *Chest*, [Epub ahead of print].

Demeester, T. R., Johnson, L. F., & Kent, A. H. (1974). Evaluation of current operations for the prevention of gastroesophageal reflux. *Ann Surg, 180*(4), 511-525.

Demeter, P., Visy, K. V., Gyulai, N., Sike, R., Toth, T. G., Novak, J., & Magyar, P. (2004). Severity of gastroesophageal reflux disease influences daytime somnolence: A clinical study of 134 patients underwent upper panendoscopy. *World J Gastroenterol, 10*(12), 1798-1801.

Dent, J., Becher, A., Sung, J., Zou, D., Agreus, L., & Bazzoli, F. (2012). Systematic review: Patterns of reflux-induced symptoms and esophageal endoscopic findings in large-scale surveys. *Clin Gastroenterol Hepatol, 10*(8), 863-873 e863.

Dent, J., El-Serag, H. B., Wallander, M. A., & Johansson, S. (2005). Epidemiology of gastro-oesophageal reflux disease: A systematic review. *Gut, 54*(5), 710-717.

El-Serag, H. B., & Sonnenberg, A. (1997). Comorbid occurrence of laryngeal or pulmonary disease with esophagitis in United States military veterans. *Gastroenterology, 113*(3), 755-760.

El-Serag, H. B., Sweet, S., Winchester, C. C., & Dent, J. (2014).

Update on the epidemiology of gastro-oesophageal reflux disease: A systematic review. *Gut, 63*(6), 871-880.

Elabiad, M. T., & Zhang, J. (2011). Detection of pepsinogen in the neonatal lung and stomach by immunohistochemistry. *J Pediatr Gastroenterol Nutr, 53*(4), 401-403.

Epstein, L. J., Kristo, D., Strollo, P. J., Friedman, N., Malhotra, A., Patil, S. P., Ramar, K., Rogers, R., Schwab, R. J., Weaver, E. M., Weinstein, M. D., & Med, A. A. S. (2009). Clinical Guideline for the Evaluation, Management and Long-term Care of Obstructive Sleep Apnea in Adults. *Journal of Clinical Sleep Medicine, 5*(3), 263-276.

Ermis, F., Akyuz, F., Arici, S., Uyanikoglu, A., Yakar, F., Pinarbasi, B., Demir, K., Ozdil, S., Besisik, F., Kaymakoglu, S., Boztas, G., Cuhadaroglu, C., & Mungan, Z. (2011). Effect of proton pump inhibitor (PPI) treatment in obstructive sleep apnea syndrome: An esophageal impedance-pHmetry study. *Hepatogastroenterology, 58*(110-111), 1566-1573.

Erpenbeck, V. J., Schmidt, R., Gunther, A., Krug, N., & Hohlfeld, J. M. (2006). Surfactant protein levels in bronchoalveolar lavage after segmental allergen challenge in patients with asthma. *Allergy, 61*(5), 598-604.

Ervine, E., McMaster, C., McCallion, W., & Shields, M. D. (2009). Pepsin measured in induced sputum--A test for pulmonary aspiration in children? *J Pediatr Surg, 44*(10), 1938-1941.

Esther, C. R., Jr., Boysen, G., Olsen, B. M., Collins, L. B., Ghio, A. J., Swenberg, J. W., & Boucher, R. C. (2009). Mass spectrometric analysis of biomarkers and dilution markers in exhaled breath condensate reveals elevated purines in asthma and cystic fibrosis. *Am J Physiol Lung Cell Mol Physiol, 296*(6), L987-993.

Fontana, G. A., & Pistolesi, M. (2003). Cough. 3: Chronic cough and gastro-oesophageal reflux. *Thorax, 58*(12), 1092-1095.

Ford, C. N. (2005). Evaluation and management of laryngopharyngeal reflux. *Jama, 294*(12), 1534-1540.

Friberg, D., Ansved, T., Borg, K., Carlsson-Nordlander, B., Larsson, H., & Svanborg, E. (1998). Histological indications of a

progressive snorers disease in an upper airway muscle. *Am J Respir Crit Care Med, 157*(2), 586-593.

Gerson, K. D., Foster, C. D., Zhang, P., Zhang, Z., Rosenblatt, M. M., & Guttentag, S. H. (2008). Pepsinogen C proteolytic processing of surfactant protein B. *J Biol Chem, 283*(16), 10330-10338.

Gerson, L. B., & Fass, R. (2009). A systematic review of the definitions, prevalence, and response to treatment of nocturnal gastroesophageal reflux disease. *Clin Gastroenterol Hepatol, 7*(4), 372-378; quiz 367.

Ghasemi, A., & Zahediasl, S. (2012). Normality tests for statistical analysis: A guide for non-statisticians. *Int J Endocrinol Metab, 10*(2), 486-489.

Gibson, P. G., Henry, R. L., & Coughlan, J. L. (2003). Gastro-oesophageal reflux treatment for asthma in adults and children. *Cochrane Database Syst Rev*(2), CD001496.

Gislason, T., Janson, C., Vermeire, P., Plaschke, P., Bjornsson, E., Gislason, D., & Boman, G. (2002). Respiratory symptoms and nocturnal gastroesophageal reflux: A population-based study of young adults in three European countries. *Chest, 121*(1), 158-163.

Goto, H., Mitsuhashi, A., & Nishioka, Y. (2014). Role of surfactant protein A in non-infectious lung diseases. *J Med Invest, 61*(1-2), 1-6.

Grabowski, M., Kasran, A., Seys, S., Pauwels, A., Medrala, W., Dupont, L., Panaszek, B., & Bullens, D. (2011). Pepsin and bile acids in induced sputum of chronic cough patients. *Respir Med, 105*(8), 1257-1261.

Green, B. T., Broughton, W. A., & O'Connor, J. B. (2003). Marked improvement in nocturnal gastroesophageal reflux in a large cohort of patients with obstructive sleep apnea treated with continuous positive airway pressure. *Arch Intern Med, 163*(1), 41-45.

Guda, N., Partington, S., & Vakil, N. (2004). Symptomatic gastro-oesophageal reflux, arousals and sleep quality in patients undergoing polysomnography for possible obstructive sleep apnoea. *Aliment Pharmacol Ther, 20*(10), 1153-1159.

Guilleminault, C., Stoohs, R., Clerk, A., Cetel, M., & Maistros, P. (1993). A cause of excessive daytime sleepiness. The upper airway resistance syndrome. *Chest, 104*(3), 781-787.

Gunnbjornsdottir, M. I., Omenaas, E., Gislason, T., Norrman, E., Olin, A. C., Jogi, R., Jensen, E. J., Lindberg, E., Bjornsson, E., Franklin, K., Janson, C., Gulsvik, A., Laerum, B., Svanes, C., Toren, K., Tunsater, A., Lillienberg, L., Gislason, D., Blondal, T., Bjornsdottir, U. S., Jorundsdottir, K. B., Talvik, R., Forsberg, B., Lundback, B., Soderberg, M., Ledin, M. C., Boman, G., Norback, D., Wieslander, G., Spetz-Nystrom, U., Cashelunge, K. S., & Ryden, E. (2004). Obesity and nocturnal gastro-oesophageal reflux are related to onset of asthma and respiratory symptoms. *Eur Respir J, 24*(1), 116-121.

Gustavsson, S., Adami, H. O., Loof, L., Nyberg, A., & Nyren, O. (1983). Rapid healing of duodenal ulcers with omeprazole: Double-blind dose-comparative trial. *Lancet, 2*(8342), 124-125.

Hallgren, K. A. (2012). Computing Inter-Rater Reliability for Observational Data: An Overview and Tutorial. *Tutor Quant Methods Psychol, 8*(1), 23-34.

Hankinson, J. L., Odencrantz, J. R., & Fedan, K. B. (1999a). Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med, 159*(1), 179-187.

Hankinson, J. L., Odencrantz, J. R., & Fedan, K. B. (1999b). Spirometric reference values from a sample of the general US population. *American Journal of Respiratory and Critical Care Medicine, 159*(1), 179-187.

Haslam, P. L., & Baughman, R. P. (1999). Report of ERS Task Force: guidelines for measurement of acellular components and standardization of BAL. *Eur Respir J, 14*(2), 245-248.

Havemann, B. D., Henderson, C. A., & El-Serag, H. B. (2007). The association between gastro-oesophageal reflux disease and asthma: A systematic review. *Gut, 56*(12), 1654-1664.

Hekking, P. P., Wener, R. R., Amelink, M., Zwinderman, A. H., Bouvy, M. L., & Bel, E. H. (2015). The prevalence of severe refractory asthma. *J Allergy Clin Immunol, 135*(4), 896-902.

Hemmink, G. J., Bredenoord, A. J., Weusten, B. L., Monkelbaan, J. F.,

Timmer, R., & Smout, A. J. (2008). Esophageal pH-impedance monitoring in patients with therapy-resistant reflux symptoms: 'On' or 'off' proton pump inhibitor? *Am J Gastroenterol, 103*(10), 2446-2453.

Hicks, D. M., Ours, T. M., Abelson, T. I., Vaezi, M. F., & Richter, J. E. (2002). The prevalence of hypopharynx findings associated with gastroesophageal reflux in normal volunteers. *J Voice, 16*(4), 564-579.

Holbrook, J. T., Wise, R. A., Gold, B. D., Blake, K., Brown, E. D., Castro, M., Dozor, A. J., Lima, J. J., Mastronarde, J. G., Sockrider, M. M., & Teague, W. G. (2012). Lansoprazole for children with poorly controlled asthma: A randomized controlled trial. *Jama, 307*(4), 373-381.

Hu, Z., Wu, J., Wang, Z., Zhang, Y., Liang, W., & Yan, C. (2015). Outcome of Stretta radiofrequency and fundoplication for GERD-related severe asthmatic symptoms. *Front Med, 9*(4), 437-443.

Iber, C., Ancoli-Israel, S., Chesson, A. L., & Quan, S. F. (2007). *The AASM manual for the scoring of sleep and associated events: Rules, terminology, and technical specifications* (1st ed.). Westchester, Illinois: American Academy of Sleep Medicine.

Janson, C., De Backer, W., Gislason, T., Plaschke, P., Bjornsson, E., Hetta, J., Kristbjarnarson, H., Vermeire, P., & Boman, G. (1996). Increased prevalence of sleep disturbances and daytime sleepiness in subjects with bronchial asthma: A population study of young adults in three European countries. *Eur Respir J, 9*(10), 2132-2138.

Janson, C., de Marco, R., Accordini, S., Almar, E., Bugiani, M., Carolei, A., Cazzoletti, L., Cerveri, I., Corsico, A., Duran-Tauleria, E., Gislason, D., Gulsvik, A., Jogi, R., Marinoni, A., Martinez-Moratalla, J., Pin, I., Vermeire, P., & Jarvis, D. (2005). Changes in the use of anti-asthmatic medication in an international cohort. *Eur Respir J, 26*(6), 1047-1055.

Janson, C., Gislason, T., De Backer, W., Plaschke, P., Bjornsson, E., Hetta, J., Kristbjarnason, H., Vermeire, P., & Boman, G. (1995). Daytime sleepiness, snoring and gastro-oesophageal reflux amongst young adults in three European countries. *J*

Intern Med, 237(3), 277-285.

Jiang, A., Liang, M., Su, Z., Chai, L., Lei, W., Wang, Z., Wang, A., Wen, W., & Chen, M. (2011). Immunohistochemical detection of pepsin in laryngeal mucosa for diagnosing laryngopharyngeal reflux. *Laryngoscope, 121*(7), 1426-1430.

Johnson, L. F., & Demeester, T. R. (1974). Twenty-four-hour pH monitoring of the distal esophagus. A quantitative measure of gastroesophageal reflux. *Am J Gastroenterol, 62*(4), 325-332.

Johnsson, F., Weywadt, L., Solhaug, J. H., Hernqvist, H., & Bengtsson, L. (1998). One-week omeprazole treatment in the diagnosis of gastro-oesophageal reflux disease. *Scand J Gastroenterol, 33*(1), 15-20.

Johnston, N., Wells, C. W., Blumin, J. H., Toohill, R. J., & Merati, A. L. (2007). Receptor-mediated uptake of pepsin by laryngeal epithelial cells. *Ann Otol Rhinol Laryngol, 116*(12), 934-938.

Johnston, N., Yan, J. C., Hoekzema, C. R., Samuels, T. L., Stoner, G. D., Blumin, J. H., & Bock, J. M. (2012). Pepsin promotes proliferation of laryngeal and pharyngeal epithelial cells. *Laryngoscope, 122*(6), 1317-1325.

Jones, P. W., Lamarca, R., Chuecos, F., Singh, D., Agusti, A., Bateman, E. D., de Miquel, G., Caracta, C., & Garcia Gil, E. (2014). Characterisation and impact of reported and unreported exacerbations: Results from ATTAIN. *Eur Respir J, 44*(5), 1156-1165.

Jovov, B., Que, J., Tobey, N. A., Djukic, Z., Hogan, B. L., & Orlando, R. C. (2011). Role of E-cadherin in the pathogenesis of gastroesophageal reflux disease. *Am J Gastroenterol, 106*(6), 1039-1047.

Kahrilas, P. J. (2010). Obstructive sleep apnea and reflux disease: Bedfellows at best. *Chest, 137*(4), 747-748.

Kawamura, O., Aslam, M., Rittmann, T., Hofmann, C., & Shaker, R. (2004). Physical and pH properties of gastroesophagopharyngeal refluxate: A 24-hour simultaneous ambulatory impedance and pH monitoring study. *Am J Gastroenterol, 99*(6), 1000-1010.

Kerr, P., Shoenut, J. P., Millar, T., Buckle, P., & Kryger, M. H. (1992).

Nasal CPAP reduces gastroesophageal reflux in obstructive sleep apnea syndrome. *Chest, 101*(6), 1539-1544.

Kiljander, T. O., Junghard, O., Beckman, O., & Lind, T. (2010). Effect of esomeprazole 40 mg once or twice daily on asthma: A randomized, placebo-controlled study. *Am J Respir Crit Care Med, 181*(10), 1042-1048.

Kiljander, T. O., Salomaa, E. R., Hietanen, E. K., & Terho, E. O. (2000). Chronic cough and gastro-oesophageal reflux: A double-blind placebo-controlled study with omeprazole. *Eur Respir J, 16*(4), 633-638.

Kindt, S., Imschoot, J., & Tack, J. (2011). Prevalence of and impact of pantoprazole on nocturnal heartburn and associated sleep complaints in patients with erosive esophagitis. *Dis Esophagus, 24*(8), 531-537.

Kitz, R., Boehles, H. J., Rosewich, M., & Rose, M. A. (2012). Lipid-Laden Alveolar Macrophages and pH Monitoring in Gastroesophageal Reflux-Related Respiratory Symptoms. *Pulm Med, 2012*, ID 673637 [Epub].

Klauser, A. G., Schindlbeck, N. E., & Muller-Lissner, S. A. (1990). Symptoms in gastro-oesophageal reflux disease. *Lancet, 335*(8683), 205-208.

Knauer-Fischer, S., & Ratjen, F. (1999). Lipid-laden macrophages in bronchoalveolar lavage fluid as a marker for pulmonary aspiration. *Pediatr Pulmonol, 27*(6), 419-422.

Kupczyk, M., ten Brinke, A., Sterk, P. J., Bel, E. H., Papi, A., Chanez, P., Nizankowska-Mogilnicka, E., Gjomarkaj, M., Gaga, M., Brusselle, G., Dahlen, B., & Dahlen, S. E. (2014). Frequent exacerbators--A distinct phenotype of severe asthma. *Clin Exp Allergy, 44*(2), 212-221.

Kuribayashi, S., Kusano, M., Kawamura, O., Shimoyama, Y., Maeda, M., Hisada, T., Ishizuka, T., Dobashi, K., & Mori, M. (2010). Mechanism of gastroesophageal reflux in patients with obstructive sleep apnea syndrome. *Neurogastroenterol Motil, 22*(6), 611-e172.

Lacy, B. E., Weiser, K., Chertoff, J., Fass, R., Pandolfino, J. E., Richter, J. E., Rothstein, R. I., Spangler, C., & Vaezi, M. F.

(2010). The Diagnosis of Gastroesophageal Reflux Disease. *Am J Med, 123*(7), 583-592.

Larsson, P., Mirgorodskaya, E., Samuelsson, L., Bake, B., Almstrand, A. C., Bredberg, A., & Olin, A. C. (2012). Surfactant protein A and albumin in particles in exhaled air. *Respir Med, 106*(2), 197-204.

Ledford, J. G., Addison, K. J., Foster, M. W., & Que, L. G. (2014). Eosinophil-associated lung diseases. A cry for surfactant proteins A and D help? *Am J Respir Cell Mol Biol, 51*(5), 604-614.

Lee, S. A., Amis, T. C., Byth, K., Larcos, G., Kairaitis, K., Robinson, T. D., & Wheatley, J. R. (2008). Heavy snoring as a cause of carotid artery atherosclerosis. *Sleep, 31*(9), 1207-1213.

Liang, W. T., Yan, C., Wang, Z. G., Wu, J. M., Hu, Z. W., Zhan, X. L., Wang, F., Ma, S. S., & Chen, M. P. (2015). Early and Midterm Outcome After Laparoscopic Fundoplication and a Minimally Invasive Endoscopic Procedure in Patients with Gastroesophageal Reflux Disease: A Prospective Observational Study. *J Laparoendosc Adv Surg Tech A, 25*(8), 657-661.

Lotvall, J., Ekerljung, L., Ronmark, E. P., Wennergren, G., Linden, A., Ronmark, E., Toren, K., & Lundback, B. (2009). West Sweden Asthma Study: Prevalence trends over the last 18 years argues no recent increase in asthma. *Respir Res, 10*, 94.

Ludviksdottir, D., Bjornsson, E., Janson, C., & Boman, G. (1996). Habitual coughing and its associations with asthma, anxiety, and gastroesophageal reflux. *Chest, 109*(5), 1262-1268.

Lugaresi, M., Aramini, B., Daddi, N., Baldi, F., & Mattioli, S. (2015). Effectiveness of antireflux surgery for the cure of chronic cough associated with gastroesophageal reflux disease. *World J Surg, 39*(1), 208-215.

Madan, J. C., Koestler, D. C., Stanton, B. A., Davidson, L., Moulton, L. A., Housman, M. L., Moore, J. H., Guill, M. F., Morrison, H. G., Sogin, M. L., Hampton, T. H., Karagas, M. R., Palumbo, P. E., Foster, J. A., Hibberd, P. L., & O'Toole, G. A. (2012). Serial analysis of the gut and respiratory microbiome in cystic fibrosis in infancy: Interaction between intestinal and respiratory tracts and impact of nutritional exposures. *MBio, 3*(4).

Maher, M. M., & Darwish, A. A. (2010). Study of respiratory disorders in endoscopically negative and positive gastroesophageal reflux disease. *Saudi J Gastroenterol, 16*(2), 84-89.

Mainie, I., Tutuian, R., Agrawal, A., Adams, D., & Castell, D. O. (2006). Combined multichannel intraluminal impedance-pH monitoring to select patients with persistent gastro-oesophageal reflux for laparoscopic Nissen fundoplication. *Br J Surg, 93*(12), 1483-1487.

Maislin, G., Pack, A. I., Kribbs, N. B., Smith, P. L., Schwartz, A. R., Kline, L. R., Schwab, R. J., & Dinges, D. F. (1995). A survey screen for prediction of apnea. *Sleep, 18*(3), 158-166.

Martinez, C. H., Okajima, Y., Murray, S., Washko, G. R., Martinez, F. J., Silverman, E. K., Lee, J. H., Regan, E. A., Crapo, J. D., Curtis, J. L., Hatabu, H., & Han, M. K. (2014). Impact of self-reported gastroesophageal reflux disease in subjects from COPDGene cohort. *Respir Res, 15*, 62.

Mertens, V., Blondeau, K., Van Oudenhove, L., Vanaudenaerde, B., Vos, R., Farre, R., Pauwels, A., Verleden, G., Van Raemdonck, D., Sifrim, D., & Dupont, L. J. (2011). Bile acids aspiration reduces survival in lung transplant recipients with BOS despite azithromycin. *Am J Transplant, 11*(2), 329-335.

Mincheva, R., Ekerljung, L., Bjerg, A., Axelsson, M., Popov, T. A., Lundback, B., & Lotvall, J. (2014). Frequent cough in unsatisfactory controlled asthma--Results from the population-based West Sweden Asthma study. *Respir Res, 15*, 79.

Mise, K., Capkun, V., Jurcev-Savicevic, A., Sundov, Z., Bradaric, A., & Mladinov, S. (2010). The influence of gastroesophageal reflux in the lung: A case-control study. *Respirology, 15*(5), 837-842.

Moayyedi, P., & Talley, N. J. (2006). Gastro-oesophageal reflux disease. *Lancet, 367*(9528), 2086-2100.

Morice, A. H., Millqvist, E., Belvisi, M. G., Bieksiene, K., Birring, S. S., Chung, K. F., Dal Negro, R. W., Dicpinigaitis, P., Kantar, A., McGarvey, L. P., Pacheco, A., Sakalauskas, R., & Smith, J. A. (2014). Expert opinion on the cough hypersensitivity syndrome in respiratory medicine. *Eur Respir J, 44*(5), 1132-1148.

Nguyen, H. N., Silny, J., & Matern, S. (1999). Multiple intraluminal electrical impedancometry for recording of upper gastrointestinal motility: Current results and further implications. *Am J Gastroenterol, 94*(2), 306-317.

Niklasson, A., Bajor, A., Bergendal, L., Simren, M., Strid, H., & Bjornsson, E. (2003). Overuse of acid suppressive therapy in hospitalised patients with pulmonary diseases. *Respir Med, 97*(10), 1143-1150.

Orr, W. (2010). Review article: Sleep-related gastro-oesophageal reflux as a distinct clinical entity. *Alimentary Pharmacology & Therapeutics, 31*(1), 47-56.

Orr, W. C., Elsenbruch, S., Harnish, M. J., & Johnson, L. F. (2000). Proximal migration of esophageal acid perfusions during waking and sleep. *Am J Gastroenterol, 95*(1), 37-42.

Orr, W. C., Goodrich, S., Estep, M. E., & Shepherd, K. (2014). The relationship between complaints of night-time heartburn and sleep-related gastroesophageal reflux. *Dis Esophagus, 27*(4), 303-310.

Orr, W. C., Johnson, L. F., & Robinson, M. G. (1984). Effect of sleep on swallowing, esophageal peristalsis, and acid clearance. *Gastroenterology, 86*(5 Pt 1), 814-819.

Orr, W. C., Robert, J. J., Houck, J. R., Giddens, C. L., & Tawk, M. M. (2009). The effect of acid suppression on upper airway anatomy and obstruction in patients with sleep apnea and gastroesophageal reflux disease. *J Clin Sleep Med, 5*(4), 330-334.

Owens, R. L., Eckert, D. J., Yeh, S. Y., & Malhotra, A. (2008). Upper airway function in the pathogenesis of obstructive sleep apnea: A review of the current literature. *Curr Opin Pulm Med, 14*(6), 519-524.

Pandolfino, J. E., & Vela, M. F. (2009). Esophageal-reflux monitoring. *Gastrointest Endosc, 69*(4), 917-930, 930 e911.

Partinen, M., & Gislason, T. (1995). Basic Nordic Sleep Questionnaire (BNSQ): A quantitated measure of subjective sleep complaints. *J Sleep Res, 4*(S1), 150-155.

Patel, A., Sayuk, G. S., & Gyawali, C. P. (2014). Acid-based

parameters on pH-impedance testing predict symptom improvement with medical management better than impedance parameters. *Am J Gastroenterol, 109*(6), 836-844.

Patterson, R. N., Johnston, B. T., Ardill, J. E., Heaney, L. G., & McGarvey, L. P. (2007). Increased tachykinin levels in induced sputum from asthmatic and cough patients with acid reflux. *Thorax, 62*(6), 491-495.

Pauwels, R. A., Buist, A. S., Calverley, P. M., Jenkins, C. R., & Hurd, S. S. (2001). Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop Summary. *Am J Respir Crit Care Med, 163*(5), 1256-1276.

Payne, R. J., Kost, K. M., Frenkiel, S., Zeitouni, A. G., Sejean, G., Sweet, R. C., Naor, N., Hernandez, L., & Kimoff, R. J. (2006). Laryngeal inflammation assessed using the reflux finding score in obstructive sleep apnea. *Otolaryngol Head Neck Surg, 134*(5), 836-842.

Perng, D. W., Chang, K. T., Su, K. C., Wu, Y. C., Wu, M. T., Hsu, W. H., Tsai, C. M., & Lee, Y. C. (2007). Exposure of airway epithelium to bile acids associated with gastroesophageal reflux symptoms: A relation to transforming growth factor-beta1 production and fibroblast proliferation. *Chest, 132*(5), 1548-1556.

Phelps, D. S., Umstead, T. M., Mejia, M., Carrillo, G., Pardo, A., & Selman, M. (2004). Increased surfactant protein-A levels in patients with newly diagnosed idiopathic pulmonary fibrosis. *Chest, 125*(2), 617-625.

Qiu, Z., Yu, L., Xu, S., Liu, B., Zhao, T., & Lu, H. (2011). Cough reflex sensitivity and airway inflammation in patients with chronic cough due to non-acid gastro-oesophageal reflux. *Respirology, 16*(4), 645-652.

Quanjer, P. H., Tammeling, G. J., Cotes, J. E., Pedersen, O. F., Peslin, R., & Yernault, J. C. (1993). Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur*

Respir J Suppl, 16, 5-40.

Rassameehiran, S., Klomjit, S., Hosiriluck, N., & Nugent, K. (2016). Meta-analysis of the effect of proton pump inhibitors on obstructive sleep apnea symptoms and indices in patients with gastroesophageal reflux disease. *Proc (Bayl Univ Med Cent), 29*(1), 3-6.

Rayner, E. B. (1933). Heartburn in Pregnancy. *Br Med J, 2*(3803), 970-971.

Reder, N. P., Davis, C. S., Kovacs, E. J., & Fisichella, P. M. (2014). The diagnostic value of gastroesophageal reflux disease (GERD) symptoms and detection of pepsin and bile acids in bronchoalveolar lavage fluid and exhaled breath condensate for identifying lung transplantation patients with GERD-induced aspiration. *Surg Endosc, 28*(6), 1794-1800.

Ribolsi, M., Savarino, E., De Bortoli, N., Balestrieri, P., Furnari, M., Martinucci, I., Casale, M., Greco, F., Salvinelli, F., Savarino, V., Marchi, S., & Cicala, M. (2014). Reflux pattern and role of impedance-pH variables in predicting PPI response in patients with suspected GERD-related chronic cough. *Aliment Pharmacol Ther, 40*(8), 966-973.

Richter, J. E., & Castell, D. O. (Eds.). (2012). *The esophagus* (5th ed.): Blackwell Publishing ltd.

Rosen, R., Johnston, N., Hart, K., Khatwa, U., & Nurko, S. (2012). The presence of pepsin in the lung and its relationship to pathologic gastro-esophageal reflux. *Neurogastroenterol Motil, 24*(2), 129-133, e84-85.

Rosias, P. (2012). Methodological aspects of exhaled breath condensate collection and analysis. *J Breath Res, 6*(2), ID 027102 [Epub].

Rothman, M., Farup, C., Stewart, W., Helbers, L., & Zeldis, J. (2001). Symptoms associated with gastroesophageal reflux disease: Development of a questionnaire for use in clinical trials. *Dig Dis Sci, 46*(7), 1540-1549.

Ruigomez, A., Rodriguez, L. A., Wallander, M. A., Johansson, S., Thomas, M., & Price, D. (2005). Gastroesophageal reflux disease and asthma: A longitudinal study in UK general

practice. *Chest, 128*(1), 85-93.

Sacco, O., Silvestri, M., Sabatini, F., Sale, R., Moscato, G., Pignatti, P., Mattioli, G., & Rossi, G. A. (2006). IL-8 and airway neutrophilia in children with gastroesophageal reflux and asthma-like symptoms. *Respir Med, 100*(2), 307-315.

Samelson, C. F. (1989). Gastroesophageal reflux and obstructive sleep apnea. *Sleep, 12*(5), 475-476.

Sasaki, T., Nakayama, K., Yasuda, H., Yoshida, M., Asamura, T., Ohrui, T., Arai, H., Araya, J., Kuwano, K., & Yamaya, M. (2009). A randomized, single-blind study of lansoprazole for the prevention of exacerbations of chronic obstructive pulmonary disease in older patients. *J Am Geriatr Soc, 57*(8), 1453-1457.

Shaaban, R., Leynaert, B., Soussan, D., Anto, J. M., Chinn, S., de Marco, R., Garcia-Aymerich, J., Heinrich, J., Janson, C., Jarvis, D., Sunyer, J., Svanes, C., Wjst, M., Burney, P. G., Neukirch, F., & Zureik, M. (2007). Physical activity and bronchial hyperresponsiveness: European Community Respiratory Health Survey II. *Thorax, 62*(5), 403-410.

Shaw, M. J., Talley, N. J., Beebe, T. J., Rockwood, T., Carlsson, R., Adlis, S., Fendrick, A. M., Jones, R., Dent, J., & Bytzer, P. (2001). Initial validation of a diagnostic questionnaire for gastroesophageal reflux disease. *Am J Gastroenterol, 96*(1), 52-57.

Shepherd, K., Hillman, D., Holloway, R., & Eastwood, P. (2011). Mechanisms of nocturnal gastroesophageal reflux events in obstructive sleep apnea. *Sleep Breath, 15*(3), 561-570.

Shepherd, K. L., Holloway, R. H., Hillman, D. R., & Eastwood, P. R. (2007). The impact of continuous positive airway pressure on the lower esophageal sphincter. *Am J Physiol Gastrointest Liver Physiol, 292*(5), G1200-1205.

Shimizu, Y., Dobashi, K., Zhao, J. J., Kawata, T., Ono, A., Yanagitani, N., Kaira, K., Utsugi, M., Hisada, T., Ishizuka, T., & Mori, M. (2007). Proton pump inhibitor improves breath marker in moderate asthma with gastroesophageal reflux disease. *Respiration, 74*(5), 558-564.

Sifrim, D., Castell, D., Dent, J., & Kahrilas, P. J. (2004). Gastro-oesophageal reflux monitoring: Review and consensus report on detection and definitions of acid, non-acid, and gas reflux. *Gut, 53*(7), 1024-1031.

Sifrim, D., Dupont, L., Blondeau, K., Zhang, X., Tack, J., & Janssens, J. (2005). Weakly acidic reflux in patients with chronic unexplained cough during 24 hour pressure, pH, and impedance monitoring. *Gut, 54*(4), 449-454.

Sifrim, D., & Zerbib, F. (2012). Diagnosis and management of patients with reflux symptoms refractory to proton pump inhibitors. *Gut, 61*(9), 1340-1354.

Silny, J. (1991). Intraluminal multiple electric impedance procedure for measurement of gastrointestinal motility. *Neurogastroenterol Motil, 3*(3), 151-162.

Simpson, J. L., Baines, K. J., Ryan, N., & Gibson, P. G. (2014). Neutrophilic asthma is characterised by increased rhinosinusitis with sleep disturbance and GERD. *Asian Pac J Allergy Immunol, 32*(1), 66-74.

Smith, J. A., Decalmer, S., Kelsall, A., McGuinness, K., Jones, H., Galloway, S., Woodcock, A., & Houghton, L. A. (2010). Acoustic cough-reflux associations in chronic cough: Potential triggers and mechanisms. *Gastroenterology, 139*(3), 754-762.

Sontag, S. J. (2000). Gastroesophageal reflux disease and asthma. *J Clin Gastroenterol, 30*(3 Suppl), S9-30.

Spiegel, B. M., Roberts, L., Mody, R., Harding, G., Kothari-Talwar, S., Kahrilas, P. J., Camilleri, M. L., Dabbous, O., & Revicki, D. A. (2010). The development and validation of a Nocturnal Gastro-oesophageal Reflux Disease Symptom Severity and Impact Questionnaire for adults. *Aliment Pharmacol Ther, 32*(4), 591-602.

Starosta, V., Kitz, R., Hartl, D., Marcos, V., Reinhardt, D., & Griese, M. (2007). Bronchoalveolar pepsin, bile acids, oxidation, and inflammation in children with gastroesophageal reflux disease. *Chest, 132*(5), 1557-1564.

Suissa, S., Dell'Aniello, S., & Ernst, P. (2012). Long-term natural history of chronic obstructive pulmonary disease: Severe

exacerbations and mortality. *Thorax, 67*(11), 957-963.

Suzuki, M., Saigusa, H., Kurogi, R., Yamamoto, T., Ishiguro, T., Yohsizawa, T., Kuyama, Y., & Furukawa, T. (2010). Arousals in obstructive sleep apnea patients with laryngopharyngeal and gastroesophageal reflux. *Sleep Med, 11*(4), 356-360.

Svensson, M., Franklin, K. A., Theorell-Haglow, J., & Lindberg, E. (2008). Daytime sleepiness relates to snoring independent of the apnea-hypopnea index in women from the general population. *Chest, 134*(5), 919-924.

Sweeney, J., Patterson, C. C., Menzies-Gow, A., Niven, R. M., Mansur, A. H., Bucknall, C., Chaudhuri, R., Price, D., Brightling, C. E., & Heaney, L. G. (2016). Comorbidity in severe asthma requiring systemic corticosteroid therapy: Cross-sectional data from the Optimum Patient Care Research Database and the British Thoracic Difficult Asthma Registry. *Thorax*.

Swidnicka-Siergiejko, A., & Dabrowski, A. (2013). Prolonged 2-day esophageal pH-metry with impedance monitoring improves symptom-reflux association analysis. *Dig Dis Sci, 58*(9), 2556-2563.

Tam, S., Woodson, B. T., & Rotenberg, B. (2014). Outcome measurements in obstructive sleep apnea: Beyond the apnea-hypopnea index. *Laryngoscope, 124*(1), 337-343.

Terada, K., Muro, S., Sato, S., Ohara, T., Haruna, A., Marumo, S., Kinose, D., Ogawa, E., Hoshino, Y., Niimi, A., Terada, T., & Mishima, M. (2008). Impact of gastro-oesophageal reflux disease symptoms on COPD exacerbation. *Thorax, 63*(11), 951-955.

Thomson, A. B., Barkun, A. N., Armstrong, D., Chiba, N., White, R. J., Daniels, S., Escobedo, S., Chakraborty, B., Sinclair, P., & Van Zanten, S. J. (2003). The prevalence of clinically significant endoscopic findings in primary care patients with uninvestigated dyspepsia: The Canadian Adult Dyspepsia Empiric Treatment - Prompt Endoscopy (CADET-PE) study. *Aliment Pharmacol Ther, 17*(12), 1481-1491.

Timms, C., Thomas, P. S., & Yates, D. H. (2012). Detection of gastro-oesophageal reflux disease (GORD) in patients with obstructive

lung disease using exhaled breath profiling. *J Breath Res, 6*(1), 016003.

Tutuian, R., Mainie, I., Agrawal, A., Adams, D., & Castell, D. O. (2006). Nonacid reflux in patients with chronic cough on acid-suppressive therapy. *Chest, 130*(2), 386-391.

Vaezi, M. F. (2011). Reflux monitoring: On or off therapy? *Am J Gastroenterol, 106*(2), 183-185.

Vakil, N., van Zanten, S. V., Kahrilas, P., Dent, J., & Jones, R. (2006). The Montreal definition and classification of gastroesophageal reflux disease: A global evidence-based consensus. *Am J Gastroenterol, 101*(8), 1900-1920.

Vakil, N. B., Halling, K., Becher, A., & Ryden, A. (2013). Systematic review of patient-reported outcome instruments for gastroesophageal reflux disease symptoms. *Eur J Gastroenterol Hepatol, 25*(1), 2-14.

van Pinxteren, B., Numans, M. E., Bonis, P. A., & Lau, J. (2004). Short-term treatment with proton pump inhibitors, H2-receptor antagonists and prokinetics for gastro-oesophageal reflux disease-like symptoms and endoscopy negative reflux disease. *Cochrane Database Syst Rev*(4), CD002095.

Vollmer, W. M., Gislason, T., Burney, P., Enright, P. L., Gulsvik, A., Kocabas, A., & Buist, A. S. (2009). Comparison of spirometry criteria for the diagnosis of COPD: Results from the BOLD study. *Eur Respir J, 34*(3), 588-597.

Williams, N. H. (1941). Variable significance of heartburn. *Am J Obstet Gynaec, 42*(5), 814-819.

Willner, D. L., Hugenholtz, P., Yerkovich, S. T., Tan, M. E., Daly, J. N., Lachner, N., Hopkins, P. M., & Chambers, D. C. (2013). Reestablishment of recipient-associated microbiota in the lung allograft is linked to reduced risk of bronchiolitis obliterans syndrome. *Am J Respir Crit Care Med, 187*(6), 640-647.

Wo, J. M., Koopman, J., Harrell, S. P., Parker, K., Winstead, W., & Lentsch, E. (2006). Double-blind, placebo-controlled trial with single-dose pantoprazole for laryngopharyngeal reflux. *Am J Gastroenterol, 101*(9), 1972-1978; quiz 2169.

Wong, W. M., Bautista, J., Dekel, R., Malagon, I. B., Tuchinsky, I.,

Green, C., Dickman, R., Esquivel, R., & Fass, R. (2005). Feasibility and tolerability of transnasal/per-oral placement of the wireless pH capsule vs. traditional 24-h oesophageal pH monitoring--A randomized trial. *Aliment Pharmacol Ther*, *21*(2), 155-163.

Yadava, K., Pattaroni, C., Sichelstiel, A. K., Trompette, A., Gollwitzer, E. S., Salami, O., von Garnier, C., Nicod, L. P., & Marsland, B. J. (2015). Microbiota Promotes Chronic Pulmonary Inflammation by Enhancing IL-17A and Autoantibodies. *Am J Respir Crit Care Med*.

Yang, Y. X., Spencer, G., Schutte-Rodin, S., Brensinger, C., & Metz, D. C. (2013). Gastroesophageal reflux and sleep events in obstructive sleep apnea. *Eur J Gastroenterol Hepatol*, *25*(9), 1017-1023.

Yuceege, M., Firat, H., Sever, O., Demir, A., & Ardic, S. (2015). The effect of adding gender item to Berlin Questionnaire in determining obstructive sleep apnea in sleep clinics. *Ann Thorac Med*, *10*(1), 25-28.

Zerbib, F., des Varannes, S. B., Roman, S., Pouderoux, P., Artigue, F., Chaput, U., Mion, F., Caillol, F., Verin, E., Bommelaer, G., Ducrotte, P., Galmiche, J. P., & Sifrim, D. (2005). Normal values and day-to-day variability of 24-h ambulatory oesophageal impedance-pH monitoring in a Belgian-French cohort of healthy subjects. *Aliment Pharmacol Ther*, *22*(10), 1011-1021.

Original publications

Paper I

Paper II

Paper III

Paper IV

Paper v