

Oesophageal hypersensitivity in patients with gastro-oesophageal reflux symptoms

Prevalence and novel treatments

A thesis submitted in partial fulfilment of the requirements of the
Degree of Doctor of Philosophy

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2018

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Abstract

Background

Gastro oesophageal reflux disease (GORD) is a leading cause of morbidity and economic importance worldwide. It is currently defined by the Montreal definition as a condition, which develops when the reflux of gastric content causes troublesome symptoms or complications. This definition based on symptoms is all encompassing, and further classification is made based on macroscopic mucosal injury as seen on gastroscopy, increased distal oesophageal acid and non acid exposure, based on 24 hour pH and impedance pH testing, and reflux symptom association.

Thus, GORD may be sub classified into the following conditions – erosive reflux disease (ERD), non erosive reflux disease (NERD), reflux hypersensitivity (RH), functional heartburn (FH) and functional chest pain (FCP). Treatment of GORD is with acid suppression therapy, anti reflux therapy and pain modulation. The pathophysiology of GORD is thought to occur in a spectrum, with varying contributions from direct mucosal injury to peripheral sensitization and central sensitization.

Further efforts to phenotype GORD populations, investigate mechanisms of symptom evolution and treatments are driven by a significant proportion of patients who are refractory to currently available therapies.

Aims

The aim of this body of work was to phenotype patients with RH, the least studied subtype of GORD, to investigate the effect of ONO 8539, a novel antagonist to the Prostaglandin E 1 receptor thought to be involved in pain perception on acid induced oesophageal pain hypersensitivity in

patients with NERD, to investigate the effect of transcutaneous vagal nerve stimulation (tVNS) on an oesophageal pain model in healthy volunteers, and to investigate the effect of slow deep breathing on oesophageal pain hypersensitivity in patients with NERD.

Methods

I investigated the above aims in a retrospective cohort study on patients referred to the gastro intestinal physiology unit of the Royal London Hospital for investigation of typical GORD symptoms, a double blind placebo controlled two period cross over study in patients with NERD, a single blind sham controlled two period cross over study in healthy volunteers and single blind sham controlled parallel study in patients with NERD respectively. The first study was done as a service evaluation exercise and the latter three studies had ethical approval from the National Research and Ethics Service (NRES), QMUL Ethics and NRES respectively.

Results

I demonstrated that phenotypic characteristics in patients with RH were distinct from NERD and FH/FCP. This was the largest cohort of RH patients evaluated, and this body of work will contribute to further research on mechanisms, pathophysiology and treatments in RH. In my second study, I was not able to demonstrate an anti nociceptive effect of ONO 8539 versus placebo on oesophageal pain hypersensitivity in patients with NERD. In my third study, I was able to demonstrate an increase in anti nociceptive parasympathetic tone, and an increase in pain tolerance threshold with tVNS compared to sham stimulation in an oesophageal pain hypersensitivity model in healthy volunteers. In my final study, I was able to demonstrate an increase in parasympathetic tone, but no improvement in lag time to pain perception with a slow deep breathing protocol

compared to a sham breathing protocol in a Modified Bernstein test model of distal oesophageal acid infusion in patients with NERD.

Conclusions

This body of work improves upon current knowledge of the phenotypic characteristics of RH, adding further weight to the definition of RH as a distinct condition. tVNS and deep slow breathing were shown to increase parasympathetic tone in healthy volunteers and patients with NERD respectively. The anti nociceptive effect of raising parasympathetic tone was only demonstrated in the healthy volunteer model of oesophageal pain hypersensitivity. The performance of the MBT model used in the two patient studies was not as reliable as the healthy volunteer model, and a new oesophageal pain hypersensitivity model for patients with NERD was proposed,

Acknowledgements

I am sincerely grateful to both my supervisors, Professor Qasim Aziz and Professor Daniel Sifrim. Giants in their respective fields, their knowledge, guidance and encouragement have been invaluable during my PhD. I am equally grateful for their grace, patience, kindness and humour, especially when times were tough.

I would like to thank Dr Adam Farmer for his help, advice and encouragement. I am grateful to Dr Jafar Jafari for training me up in oesophageal physiology testing.

My colleagues, and most importantly, my friends, Laura Brockway, Joanne Ooi, Charlotte Betteridge, Alicia Green, Annika Rasijeff, Ahmed Albusoda, Chung Lee and Ron Fried – thank you for making me laugh, for the lovely cakes (Jo especially), for the ad hoc emergency childcare (Laura, Jo and Charlotte – you are definitely “the best people at mama’s work!”, although Ron will give you a run for your money with his Minion Frustration skills!) and for generally making the Wingate the best place I have ever worked at. Thank you to Shirley, Dealhya and Etsuro in the GI physiology unit and Sam and Lynn in the Wingate office. Iona Bell, I know you didn’t think you should be in this list but your encouragement during my write up has been invaluable.

I am grateful for the love and support of my family, especially my mum.

Most of all, my son Zaki, my daughter Lilia and my husband Mourad. Your love, laughter and hugs have made this possible.

I dedicate this thesis to

Mourad, Zaki and Lilia – my unlimited suppliers of the most delicious hugs

Dr Susan Margaret Surguy – my rock at the WIngate

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Publication, abstracts, presentations and prizes relating to this thesis

- British Society of Gastroenterology's 2016 Prize for best oral presentation in the category Neurogastroenterology for presentation OC-069.
Randomized controlled trial – Transcutaneous auricular electrical vagal nerve stimulation prevents the development of acid induced esophageal hypersensitivity
- DDW 2016 - 520 Randomized Controlled Trial: Transcutaneous Electrical Vagal Nerve Stimulation Prevents the Development of Acid Induced Esophageal Hyperalgesia. Adam D. Farmer, Gehanjali Amarasinghe, Jacob Juel, Maria Moller, Geeth Silva, Christina Brock, Daniel Sifrim, Asbjorn Drewes. *Gastroenterology*, Vol. 150, Issue 4, S107. Published in issue: April 2016.
- BSG 2016 - Abstract Submission Neurogastroenterology: functional disorders, motility and clinical physiology BSG16-ABS-1814 . Postprandial intragastric pH levels are elevated for significantly longer on reflux monitoring in patients with confirmed gastroparesis. J. L. S. Ooi* 1 , G. Amarasinghe1 , K. Nikaki1 , S. Gabieta-Sonmez2 , E. Yazaki1 , D. Sifrim1 , P. Woodland1, 2 1Barts and the London School of Medicine and Dentistry, Queen Mary University of London, 2Royal London Hospital, Barts Health NHS Trust, London, United Kingdom.
- Functional oesophageal disorders - Gehanjali Amarasinghe, Daniel Sifrim. Published in : *HAMDAN MEDICAL JOURNAL* ; Vol 8, No 3 (2015). DOI : 10.7707/hmj.467.
- UEG Week 2015 - Abstract Submission Topic area: 2. OESOPHAGEAL, GASTRIC AND DUODENAL Topic: 2.14. Reflux disease, pathogenesis UEG15-ABS-5252 Acid-hypersensitive oesophagus refractory to PPI: relevance of gas-related reflux events and hypervigilance . G. Amarasinghe* 1, P. Woodland1 , J. L. S. Ooi1 , E. Yazaki1 , Q. Aziz1 , D. Sifrim1 1Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom.

- Functional Esophageal Disorders: Pharmacological Options. *Drugs* August 2014, Volume 74, Issue 12, pp 1335-1344 Date: 08 Aug 2014. Gehanjali Amarasinghe, Daniel Sifrim.
- Emerging therapeutic options in GERD - Best Practice Research in Clinical Gastroenterology. 2013 Jun; 27(3): 455-67. Woodland P, Amarasinghe G, Sifrim D

List of abbreviations

<i>5-HT</i>	<i>5-Hydroxytryptophan</i>
<i>ANS</i>	<i>Autonomic nervous system</i>
<i>ASIC</i>	<i>Acid-sensing ion channel</i>
<i>ATP</i>	<i>Adenosine triphosphate</i>
<i>BFI</i>	<i>Big five inventory</i>
<i>BMI</i>	<i>Body mass index</i>
<i>cAMP</i>	<i>Cyclic adenosine monophosphate</i>
<i>CCK</i>	<i>cholecystokinin</i>
<i>CGRP</i>	<i>Calcitonin gene-related peptide</i>
<i>CNS</i>	<i>Central nervous system</i>
<i>CI</i>	<i>Confidence interval</i>
<i>CRH</i>	<i>Corticotropin-releasing hormone</i>
<i>CVT</i>	<i>Cardiac vagal tone</i>
<i>DIS</i>	<i>Dilated intercellular spaces</i>
<i>EP1</i>	<i>Prostaglandin E1 receptor</i>
<i>ERD</i>	<i>Erosive reflux disease</i>
<i>FCP</i>	<i>Functional chest pain</i>
<i>FH</i>	<i>Functional heartburn</i>
<i>GOJ</i>	<i>Gastro-oesophageal junction</i>
<i>GORD</i>	<i>Gastro-oesophageal reflux disease</i>
<i>HADS</i>	<i>Hospital anxiety and depression score</i>
<i>H₂RA</i>	<i>Histamine-2-receptor antagonist</i>
<i>HRM</i>	<i>High resolution manometry</i>
<i>IQR</i>	<i>Interquartile range</i>
<i>LIF</i>	<i>Leukaemia inhibitory factor</i>
<i>LOS</i>	<i>Lower oesophageal sphincter</i>
<i>MBT</i>	<i>Modified Bernstein test</i>
<i>MNBI</i>	<i>Mean nocturnal baseline impedance</i>
<i>MII pH</i>	<i>Multichannel intraluminal impedance pH</i>
<i>NERD</i>	<i>Non-erosive reflux disease</i>
<i>NGF</i>	<i>Nerve growth factor</i>
<i>NMDA</i>	<i>N-methyl-D-aspartate</i>
<i>NO</i>	<i>Nitric oxide</i>
<i>NSAIDs</i>	<i>Non-steroidal anti-inflammatory drugs</i>
<i>NRES</i>	<i>National Research Ethics Service</i>
<i>NTS</i>	<i>Nucleus tractus solitarius</i>
<i>OGD</i>	<i>Oesophagogastroduodenoscopy</i>
<i>OGJ</i>	<i>Oesophago gastric junction</i>
<i>PAF</i>	<i>platelet-activating factor</i>
<i>PAR</i>	<i>Proteinase activated receptor</i>
<i>PGE</i>	<i>Prostaglandin E</i>
<i>PNS</i>	<i>Parasympathetic nervous system</i>
<i>PNS</i>	<i>Peripheral nervous system</i>

<i>PPI</i>	<i>Proton pump inhibitor</i>
<i>PSPW</i>	<i>Post swallow pressure wave</i>
<i>RH</i>	<i>Reflux hypersensitivity</i>
<i>SAP</i>	<i>Symptom associated probability</i>
<i>SEM</i>	<i>Standard error of the mean</i>
<i>SI</i>	<i>Symptom index</i>
<i>SNS</i>	<i>Sympathetic nervous system</i>
<i>TLOSR</i>	<i>Transient lower oesophageal sphincter relaxation</i>
<i>TNF</i>	<i>Tumour necrosis factor</i>
<i>TRPV</i>	<i>Transient receptor potential vanilloid</i>
<i>tVNS</i>	<i>Transcutaneous vagal nerve stimulation</i>
<i>VIP</i>	<i>Vasoactive intestinal peptide</i>

Chapter 1

Oesophageal hypersensitivity in patients with gastro-oesophageal reflux symptoms: Prevalence and novel treatments

General Introduction

In this thesis, I look at oesophageal pain in reflux hypersensitivity (RH) and non-erosive reflux disease (NERD), both of which are recognised as increasingly important entities in the field of gastro oesophageal reflux disease (GORD). GORD is characterised by pathologically increased distal oesophageal acid exposure. On endoscopy, there may be evidence of macroscopic oesophageal injury. NERD is defined as a diagnosis of GORD in the absence of macroscopic oesophageal injury (1).

RH is defined as an *increased sensitivity* of the oesophagus to *physiological* acid exposure and reflux, in the absence of any macroscopic injury (2). In this thesis, I have focussed primarily on reflux hypersensitivity and NERD, as patients with these two conditions make up a large proportion of patients who are refractory to, or only partially responsive to the current best available pharmacological therapies for GORD (proton pump inhibitors and H₂ receptor antagonists (3). In some sections of this thesis, I compared these two groups to patients with functional heartburn (FH) and functional chest pain (FCP). Patients with FH and FCP have symptoms of heartburn and chest pain, but with no evidence of oesophageal injury at oesophago gastro duodenoscopy (OGD) as well as normal oesophageal acid exposure with no significant reflux-symptom association at 24 hour pH or impedance pH studies(4).

In patients with GORD, where macroscopic injury of the oesophagus (such as oesophagitis) is evident, oesophageal injury is thought to be the cause of sensitivity to stimuli. The mechanism of oesophageal sensitivity to stimuli in patients with NERD and RH is less clear. Microscopic injury or other neurophysiological mechanisms may play a role in the development of oesophageal symptoms in all these patients, especially those with RH and NERD, and certainly there is a definite difference between patients with NERD and FH (5). In fact, it may even be a continuum, ranging from macroscopic oesophageal injury to microscopic oesophageal injury as well as neurophysiological changes, which account for hypersensitivity to mechanical, chemical and electrical stimuli. Therefore, understanding the patient with a hypersensitive oesophagus in terms of prevalence and

characteristics is an important step towards formulating novel treatment strategies in this group of patients who are refractory (completely or partially) to conventional therapies currently available.

In this thesis, in the first chapter, I briefly review the human oesophagus, considering in particular current advances of knowledge in innervation of the human oesophagus, as well as current ideas of mechanisms of oesophageal sensation. I then review typical reflux symptoms, the starting point of a patients' journey as they present to the gastroenterologist, as well as discuss GORD, NERD, RH, FH and FCP. Advances in diagnostic techniques and current thinking in terms of diagnostic algorithms and, the significant therapeutic gap currently faced by patients suffering with GORD as well as RH are discussed. I finally introduce potential novel treatment avenues –such as the treatment of oesophageal pain by modulation of the autonomic nervous system as well as pharmacological modulation of oesophageal pain.

In the second chapter, I initially focus on the prevalence of RH in our department. This will form the largest cohort of patients identified with RH studied thus far. My aim was to phenotype this population and then compare this population with patients diagnosed with NERD and those diagnosed with functional oesophageal symptoms (FH and FCP). The aim of this comparison was to assess possible avenues of therapies for RH, some of which have informed the following chapters.

In the third chapter of this thesis, I present a clinical trial of a pharmacological agent that causes blockade of prostaglandin EP1 receptors, and the effect of this agent on oesophageal pain. This was a two centre, double blinded placebo controlled two period cross over study, and the effect of the treatment agent was assessed via responses to acid stimulation in the form of a modified Bernstein test as well as change in psychological parameters.

In the fourth chapter, I focus on neurophysiological modulation of the autonomic nervous system as a possible means of treating patients with NERD and RH. Here, I investigated the effect of transcutaneous vagal nerve stimulation on acid induced oesophageal hyperalgesia in healthy adult volunteers.

In the fifth chapter, I investigated the effect of slow deep breathing (which has been previously demonstrated by our group to increase parasympathetic tone) as a means of autonomic modulation of oesophageal pain in patients with NERD in a single blinded parallel sham controlled study. The control group of patients used a type of sham breathing as their treatment intervention. Response to acid stimulation in the form of a modified Bernstein test, as well as change in psychological parameters were assessed.

In the final chapter, I summarise the body of work in this PhD, discussing the contribution that has been made towards furthering our knowledge of RH and NERD as well as the advancement of treatments for oesophageal pain. I discuss limitations encountered as well as further directions of study.

The adult human oesophagus

By adulthood, the human oesophagus is on average 18 to 26 cm measured from the upper oesophageal sphincter at the level of C5/6, to the lower oesophageal sphincter at the level of T10. It is able to distend by 2cm in the antero-posterior plane, and by 3cm in the lateral plane in the presence of a bolus. In the absence of a bolus, it appears as a compressed tubular structure, extending from the suprasternal notch to the diaphragmatic hiatus, passing posterior to the trachea, the tracheal bifurcation, and the left main stem bronchus. The aortic arch lies anterior to the oesophagus until the level of T8, where the oesophagus is shifted anterior to the descending aorta.

Macroscopically, three distinct segments are recognised – the cervical oesophagus (from the upper oesophageal sphincter to the suprasternal notch), the thoracic oesophagus (from the suprasternal notch to the diaphragmatic hiatus) and the abdominal oesophagus (from the diaphragmatic hiatus to the gastric cardia).

The upper and lower oesophageal sphincters are delineated by high pressure zones at the upper and lower ends of the oesophagus respectively. Once developed fully, the oesophageal wall consists of 4 layers from a histological perspective.

- *Mucosa* – this is the luminal layer and it is composed of nonkeratinised stratified squamous epithelium. At the gastro-oesophageal junction, this changes to columnar epithelium. The point of change is the squamocolumnar junction, also called the Z line.
- *Submucosa* – this is a layer of connective tissue, connecting the mucosa to the muscular layer below it. It contains blood vessels, submucosal nerve plexi and oesophageal glands.
- *Muscularis mucosa* – this layer is formed of circular and longitudinal muscle fibres. The inner circular muscle fibre layer is continuous with muscles in the cricopharyngeal part of the inferior constrictor muscles of the pharynx and the

oblique muscle fibres of the stomach. The outer longitudinal muscle layer forms a continuous coat around the oesophagus except posterosuperiorly, 3-4 cm below the cricoid cartilage; here, the muscle layer diverges as 2 fascicles that ascend obliquely to the anterior aspect of the oesophagus. These muscle fibres in general are striated fibres in the proximal third of the oesophagus and smooth fibres in the distal two thirds of the oesophagus.

- *Adventitia* – this is an elastic, dense, fibrous connective tissue layer covering the oesophagus. The oesophagus does not have a serosal layer in contrast to the rest of the gastrointestinal tract.

The lower oesophageal sphincter

The lower oesophageal sphincter (LOS) and the diaphragm control movement of oesophageal contents into the stomach as well as the movement of gastric contents (reflux) and vented gas (belching) into the oesophagus.

The LOS is 2 to 4 cm in length. The circular and longitudinal smooth muscle layers of the oesophagus continue into the LOS, where the circular muscles fibres become C shaped towards the lower LOS, with those arising from the left side of the LOS clasping fibres arising from the right side. The left sided fibres also combine with gastric sling fibres, which loop around the GOJ, and in turn form the oblique muscle layer of the stomach. The C shaped clasping fibres are able to maintain basal tone more effectively than the sling fibres which are more responsive to cholinergic stimulation (6). This allows for an asymmetric configuration of the LOS.

The diaphragmatic sphincter occurs as a result of the action of the crural diaphragm on the lower oesophagus during inspiration, hence the characteristic pressure inversion point recognised on high-resolution manometry. During inspiration, when gastric pressures are higher, the diaphragmatic sphincter augments the integrity of the oesophago- gastric junction (OGJ) to prevent reflux of gastric contents. The effect of the diaphragmatic sphincter can be disrupted by gastric

causes (hiatal hernias, obesity, overeating, reduced gastric fundic compliance, delayed gastric emptying) and respiratory causes of lower intrathoracic pressure (obstructive sleep apnoea and asthma), thus reducing the integrity of the LOS and increasing the likelihood of gastro oesophageal reflux disease.

Innervation of the LOS is via the vagus (parasympathetic) nerve and splanchnic (sympathetic) nerves. Vagal afferents from the LOS travel to the nucleus tractus solitarius (NTS) of the hindbrain whilst vagal efferents travel from the preganglionic fibres of the dorsal motor nucleus of the vagus to the LOS. Therefore this circuit involving the NTS and the dorsal motor nucleus controls LOS relaxation.

Neurotransmitters and hormones involved in lowering LOS tone include nitric oxide (NO) and nitrates, vasoactive intestinal peptide (VIP), nicotine, β -adrenergic agonists (1,2, and 3), dopamine (D2), cholecystokinin (CCK), secretin, calcitonin gene-related peptide (CGRP), adenosine, and prostaglandin E. LOS tone is increased by muscarinic M2 and M3 receptor agonists, gastrin, substance P, α -adrenergic agonists, and prostaglandin F2 alpha (7).

Innervation of the human oesophagus

<i>Sympathetic afferent innervation</i>	<i>Parasympathetic afferent innervation</i>	<i>Enteric innervation</i>
<p><i>Spinal afferent nerve endings in the muscle layer and serosa act as nociceptors for perception of discomfort and pain and are also sensitive to mechanical distention.</i></p> <p><i>Spinal afferent nerve endings in the mucosa are sensitive to acid-induced pain during topical exposure to intraluminal acid.</i></p> <p><i>Many of these spinal afferents contain CGRP and substance P.</i></p> <p><i>Cell bodies of the spinal afferents occur in the dorsal root ganglia and terminate in the spinal column, and in the nucleus gracilis and cuneatus in the brainstem. From there, they project, through the thalamus, to primary sensory and insular cortical areas.</i></p>	<p><i>Nerve endings in oesophageal smooth muscle layer are sensitive to mechanical distention.</i></p> <p><i>Nerve endings in the oesophageal mucosa are sensitive to various osmo-, chemo-, thermo-, and mechanical intraluminal stimuli.</i></p> <p><i>Cell bodies of vagal afferents occur in the nodose ganglia and project to the nucleus solitarius.</i></p> <p><i>Parasympathetic afferents comprise of 80% of the vagal trunk.</i></p>	<p><i>The enteric nerves in the myenteric and submucosal plexi provide the intrinsic innervation of the oesophagus.</i></p>
		<p><i>The myenteric (also known as the Auerbach's plexus) ganglia lie between the longitudinal and the circular layers of the tunica muscularis. There are more myenteric ganglia in the smooth muscle part of the oesophagus than the striated muscle part of the oesophagus.</i></p> <p><i>The myenteric plexus regulates contraction of the outer muscle layers.</i></p>
		<p><i>The submucous (also known as the Meissner's plexus) ganglia lie in the submucosa.</i></p> <p><i>The submucous plexus regulates secretion and peristaltic contractions of the muscularis mucosae.</i></p>
<i>Sympathetic efferent innervation</i>	<i>Parasympathetic efferent innervation</i>	
<p><i>Sympathetic efferents come from the cervical and the thoracic sympathetic chain (spinal segments T1–T10), regulating vascular constriction, contraction of oesophageal sphincters, relaxation of the muscular wall, and increases in glandular and peristaltic activity.</i></p>	<p><i>Parasympathetic efferents come from the nucleus ambiguus and dorsal motor nucleus of the vagus nerve, providing motor innervation to the oesophageal muscular coat and secretomotor innervation to oesophageal glands.</i></p>	<p><i>A network of fibers interconnects these two plexi.</i></p>

Table 1.1: Innervation of the oesophagus is via the enteric nervous system (ENS) as well as neurons from the sympathetic and parasympathetic ganglia. (8)

Development of innervation of the human oesophagus

Development of neural tissue in the embryo begins very early starting with the neural plate (a thickening in the ectoderm lining the base of the amniotic cavity). In the third week, the lateral ends of the neural plate fold over and join together forming the neural tube, the lumen of which is the neural canal. The cells at the leading edge of the neural fold migrate to either side of the neural tube to form the neural crest, which then lies on either side of the neural tube. The cells lining the neural tube (neuroepithelium) give rise to cells of the central nervous system (CNS) whilst neural crest cells and ectodermal placode cells give rise to cells of the peripheral nervous system (PNS).

Development of the central nervous system

The neural tube epithelium forms neurons, glial cells and ependymal cells that make up the developing brainstem and spinal cord. The neurons migrate outwards forming the mantle layer (future grey matter) with their axons pointing outwards forming the marginal layer (future white matter). The mantle layer of the brainstem and spinal cord has a dorsal “alar” plate and a ventral “basal” plate. Cranial nerve motor nuclei develop from the brainstem basal plates, and cranial nerve sensory nuclei develop from the brainstem alar plates. All the nuclei in turn are grouped into 7 longitudinal columns in the brainstem – three basal columns and four alar columns.

Three basal columns

1. *Somatic efferent (containing the nuclei of III, IV, VI and XII)*
2. *Special visceral efferent (containing nuclei of V, VII located cranially, with the nuclei for X, XI and XII supplied by the nucleus ambiguus)*
3. *General visceral efferent (containing the parasympathetic component of VII and IX as well as the dorsal nucleus of X containing the parasympathetic component of the vagus nerve supplying the viscera)*

Four alar columns

1. *General visceral afferent (contains a nucleus receiving information from IX and X)*
2. *Special visceral afferent (contains the nucleus tractus solitarius receiving information from VII, IX and X).*
3. *General somatic afferent (contains nuclei receiving information from V, VII, IX and X).*
4. *Special somatic afferent (contains the cochlear and vestibular nuclei receiving information from VIII).*

Meanwhile, neural crest cells differentiate in the cranio-caudal direction into cranial neural crest cells, vagal neural crest cells, trunk neural crest cells and sacral/lumbosacral neural crest cells. A population of cranial neural crest cells gives rise to the cranial component of the parasympathetic nervous system (CN III, VII, IX and X).

Development of the peripheral nervous system

A population of trunk neural crest cells aggregate lateral to the neural tube forming clumps of cells, which differentiate into dorsal root ganglia, which will receive sensory information from the viscera and extremities. ***Here, the beginning of the basis of central sensitization is seen as some overlap in the corresponding levels of the neural tube and the dorsal root ganglia becomes evident.***

Other trunk neural crest cells migrate to an area ventral to the dorsal root ganglia to form the chain ganglia, which eventually become the sympathetic chain, providing autonomic motor innervation to viscera as well as controlling glandular secretions, peristalsis and heart rate. These nerves develop in the intermediolateral columns of T1 to L2/L3 (thoracolumbar system), growing through the ventral root, branching off to form the white ramus, which enters the sympathetic chain at that level. Some neurons synapse with postganglionic sympathetic neurons in the sympathetic chain (these postganglionic neurons leave the sympathetic chain via the grey ramus to enter the spinal nerve), whilst others just pass through, to synapse with another sympathetic chain ganglia above or below it (providing autonomic innervation to other spinal levels other than T1 to L2/3) or, more distally in the prevertebral ganglia. The sympathetic prevertebral ganglia are formed from populations of thoracic and lumbar neural crest cells, and they develop in association with the celiac artery, superior mesenteric artery, renal arteries and the inferior mesenteric arteries, migrating with the vessel closer to their target organ.

Somatic motor axons grow out from the basal columns of the developing spinal cord forming a pair of ventral roots at the level of each somite. Autonomic neurons from the intermediolateral cell

columns join the ventral roots at the levels of T1 to L2/3. This is followed by neurons in the dorsal root ganglions growing both proximally towards the spinal cord and distally via the spinal nerves to the periphery. Considering growth in the proximal direction, when the axons reach the spinal cord, they synapse with association neurons that in turn synapse with autonomic motor neurons, somatic motor neurons, or, they ascend to higher levels in the cord where they synapse with autonomic motor or somatic motor neurons.

Preganglionic parasympathetic neurons form from a population of cranial neural crest cells and sacral neural crest cells (craniosacral system) that migrate distally to form parasympathetic ganglia close to the viscera they supply. The cranial parasympathetic fibres travel to the parasympathetic ganglia via the vagus nerve. The sacral parasympathetic fibres travel to the hindgut and pelvic visceral ganglia via the pelvic splanchnic nerves. The parasympathetic ganglia that become localised in the gut become enteric ganglia.

In addition to the above, vagal and lumbosacral neural crest cells migrate into the wall of the gut tube to form the *enteric nervous system* (ENS). Vagal neural crest cells invade the gut tube in a cranio-caudal direction from the oesophagus to the rectum whilst lumbosacral neural crest cells invade the gut tube from the rectum in a cranial direction, resulting in the terminal portion (colon/rectum) of the gut tube having ENS fibres derived from both cranial and lumbosacral neural crest cells (lack of this migration process is implicated in Hirschsprung disease). The neurons of the ENS become grouped into ganglia localised to the submucosal plexus (Meissner's plexus), which lies adjacent to the circular muscle layer, and the myenteric plexus (Auerbach's plexus), which lies between the circular and longitudinal muscle layers (with some neurons located in the lamina propria). It consists of glia, interconnected afferent and efferent neurons and interneurons. It regulates peristalsis, blood flow, secretion, absorption, and endocrine processes. It is able to function without CNS input. The interstitial cells of Cajal are involved in the integration of signals between the ENS and the smooth muscle cells of the gut. They function as pacemaker cells, driving

peristalsis in the gut. They are thought to arise from mesodermal precursors of smooth muscle cells (and not neural crest cells) (9, 10).

The sympathetic innervation of the (heart and) gut

The sympathetic supply of the heart originates from T1 to T4. Some fibres from T1 travel up the sympathetic chain to synapse with postganglionic nerves in the three cervical chain ganglia. Other fibres from T1 as well as those from T2 to T4 synapse with postganglionic nerves in the sympathetic chain, and both these sets of postganglionic fibres form the cardiac nerves. The trachea and lungs are also innervated by postganglionic fibres originating either from the sympathetic chain or cervical chain ganglia innervated by preganglionic fibres originating from T1 to T4.

The gut is supplied by preganglionic fibres from T5 to L2/3, which pass into the sympathetic chain and then leave as splanchnic nerves (without synapsing in the sympathetic chain), which synapse with postganglionic nerves in the prevertebral ganglia. These postsynaptic nerves supply the gut from the oesophagus to the anus. Therefore, fibres from T5 to T9 /10 supply the greater splanchnic nerves serving the coeliac ganglion (distal foregut), fibres from T10 and T11 supply the lesser splanchnic nerves serving the superior mesenteric (midgut and ascending colon and two thirds of the transverse colon) and aorticorenal ganglia (kidney and suprarenal gland), fibres from T12 supply the least splanchnic nerves serving the renal plexus, and fibres from L1/L2 supply the lumbar splanchnic nerves serving the inferior mesenteric ganglion plexus (hindgut, so distal third of transverse colon, descending colon, sigmoid colon and proximal 2/3 of the anal canal).

The parasympathetic innervation of the (heart and) gut

The cranial parasympathetic (efferent) ganglia (arising from neural crest cells) and the cranial sensory (afferent) ganglia (arising from neural crest cells and placode cells) start to appear in week 5. The cranial parasympathetic ganglia are the ganglia associated with the vagus nerve (supplying the gut, heart, lungs and pelvic organs) and the parasympathetic ganglia associated with III, VII and IX (supplying the relevant structures of the head).

The vagus nerve in particular contains somatic motor and sensory fibres as well as preganglionic parasympathetic fibres. Its branches subsequently supply structures of the head as well as the thorax and abdomen, to synapse with postganglionic vagal fibres at the many small parasympathetic ganglia located in the walls of the organs supplied. Parasympathetic preganglionic fibres that arise from the sacral spinal cord (S2 – S4) travel through the ventral rami and join to make up the pelvic splanchnic nerves, which supply ganglia in the descending colon, sigmoid colon, rectum, ureter, bladder and genitals. The postganglionic fibres from all these ganglia innervate smooth muscle and glands (10) (11).

The vagus nerve

The vagus nerve is longest and most complex of the cranial nerves. It contains somatic and visceral afferent fibres, as well as general and special visceral efferent fibres. The vagus nerve is a major component of the parasympathetic component of the ANS, hence stimulation of the vagus nerve is a possible means of increasing parasympathetic tone, harnessing an analgesic effect (12).

The vagus nerve exits from the medulla oblongata in the groove between the olive and the inferior cerebellar peduncle of the brain. It leaves the skull through the jugular foramen, where it has upper and lower ganglionic swellings, which are the sensory ganglia of the nerve - the superior jugular ganglion and the inferior nodose ganglion. The vagus nerve is joined by the cranial root of the accessory nerve, just below the inferior ganglion.

The meningeal branch of the vagus nerve arises at the superior ganglion and re-enters the cranium through the jugular foramen to supply the posterior fossa dura. The **auricular branch** supplies sensations to the posterior aspect of the external ear (pinna) and the **posterior part of the external auditory canal**. It arises also from the superior ganglion and enters the mastoid canaliculus in the lateral part of the jugular foramen. It exits again through the tympanomastoid suture of the temporal bone to reach the skin. It communicates with branches of the seventh (facial) and ninth (glossopharyngeal) cranial nerves.

Components	Function	Central connection	Cell bodies	Peripheral distribution
<i>Branchial motor (efferent special visceral)</i>	<i>Swallowing, phonation</i>	<i>Nucleus ambiguus</i>	<i>Nucleus ambiguus</i>	<i>Pharyngeal branches, superior and inferior laryngeal nerves</i>
<i>Visceral motor (efferent general visceral)</i>	<i>Involuntary muscle and gland control</i>	<i>Dorsal motor nucleus X</i>	<i>Dorsal motor nucleus X</i>	<i>Cardiac, pulmonary, esophageal, gastric, celiac plexuses, and muscles, and glands of the digestive tract</i>
<i>Visceral sensory (afferent general visceral)</i>	<i>Visceral sensibility</i>	<i>Nucleus tractus solitarius</i>	<i>Inferior ganglion X</i>	<i>Cervical, thoracic, abdominal fibers, and carotid and aortic bodies</i>
<i>Visceral sensory (afferent special visceral)</i>	<i>Taste</i>	<i>Nucleus tractus solitarius</i>	<i>Inferior ganglion X</i>	<i>Branches to epiglottis and taste buds</i>
<i>General sensory (afferent general somatic)</i>	<i>Cutaneous sensibility</i>	<i>Nucleus spinal tract V</i>	<i>Superior ganglion X</i>	<i>Auricular branch to external ear, meatus, and tympanic membrane</i>

Table 1.2: Summary of Central Connections, Components, Function, and Peripheral Distribution of the Vagus Nerve. Adapted from <https://emedicine.medscape.com/article/1875813-overview#a1>

The vagus nerve descends within the carotid sheath, posterolateral to the internal and common carotid arteries and medial to the internal jugular vein at the root of the neck. Branches of the vagus nerve arising at the neck are the pharyngeal branches, the superior laryngeal nerve, the recurrent laryngeal nerve and the superior cardiac nerve.

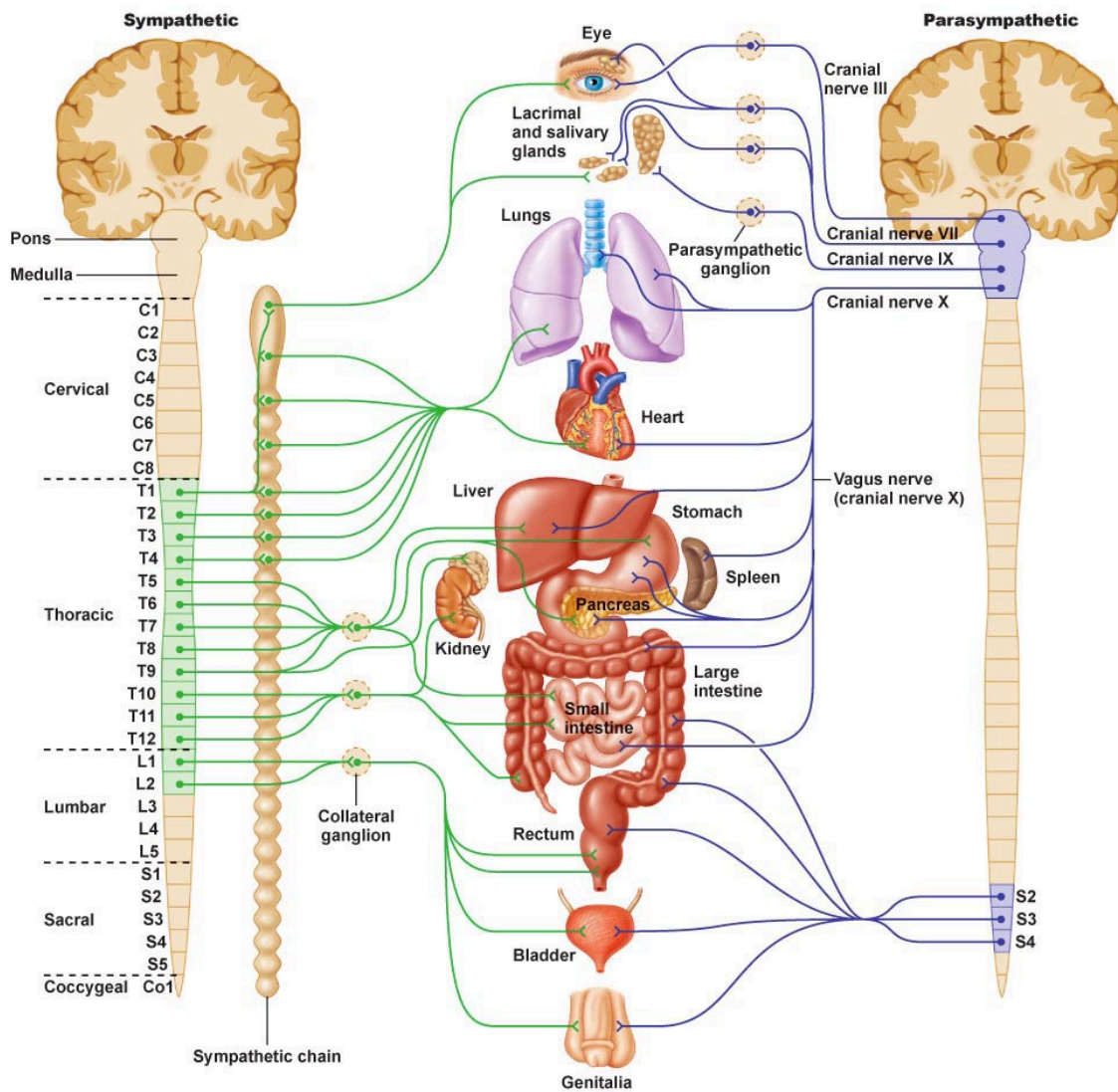
The right vagus crosses in front of the first part of the subclavian artery. It then reaches the thorax on the right side of the trachea, inclines behind the hilum of the right lung and courses medially toward the oesophagus to form the oesophageal plexus with the left vagus nerve.

The left vagus crosses in front of the left subclavian artery to enter the thorax. It descends on the left side of the aortic arch, and travels behind the phrenic nerve. It courses behind the root of the left lung to reach the oesophagus and form the oesophageal plexus by joining the opposite (right) vagus nerve.

The inferior cardiac branch on the right side arises from the trunk of the vagus as it lies beside the trachea. On the left side, it originates from the recurrent laryngeal nerve only. These branches end in the deep part of the cardiac plexus. It is important to note that the right vagus nerve is involved in innervation of the sinoatrial node of the heart whilst the left vagus nerve innervates the atrioventricular node. Experimental evidence in rats has demonstrated that stimulation of the left vagal nerve does not affect heart rate, whereas right vagal nerve stimulation does affect heart rate (13).

The anterior and posterior bronchial branches of the vagus nerve are distributed as branches on the anterior surface of the root of the lung, forming the anterior pulmonary plexus after joining branches from the sympathetic trunk. The posterior bronchial branches are larger than the anterior and lie on the posterior surface of the root of the lung to form the posterior pulmonary plexus (with contributory sympathetic fibres) as well.

The hepatic branches originate from the left vagus, and join the hepatic plexus. From here further branches are distributed to the liver. The gastric branches supply the stomach with the right vagus forming the posterior gastric plexus and the left vagus forming the anterior gastric plexus. The coeliac branches are derived mainly from the right vagus nerve, and they join the coeliac plexus and supply the pancreas, spleen, kidneys, adrenals, and intestine.



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Figure 1.1: The vagus nerve and its distribution.

Vagal nerve stimulation

Stimulation of the vagus nerve using implanted electrical stimulators is well established as a therapy for conditions such as epilepsy (14) depression (15) and gastroparesis (16). In terms of nausea, vomiting and sensitivity to distension, gastric electrical stimulation has been shown to improve symptoms in patients with gastroparesis, especially diabetic gastroparesis in the short and long term (16, 17). The evidence for vagal nerve stimulation in other conditions such as sepsis and inflammation as described in the previous paragraph, is emerging (18). In the context of pain, vagal nerve stimulation has been used to treat fibromyalgia (19) and migraine (20). Implanted electrical stimulators, bring with them complications associated with an invasive procedure.

Non invasive electrical vagal nerve stimulation is increasingly being considered, with the advent of several devices on the market of late. There is some evidence to show possible utility and effectiveness in epilepsy (21), migraine (22), nausea and gastroparesis (23) at present.

Oesophageal nociception

The uncontrolled activation of nociceptors is a problem in allodynia and hyperalgesia. Allodynia is the sensation of pain in response to a stimulus that does not usually cause pain. In the absence of a stimulus, pain is not felt. In hyperalgesia, there is an exaggerated response to noxious stimuli with persistence, despite removal of the stimuli. This persistent pain can be either nociceptive pain (activation of nociceptors in response to inflammation or injury) or neuropathic pain (direct injury to nerves in the peripheral nervous system (PNS) or central nervous system (CNS) resulting in burning or electrifying pain).

Nociceptors are free nerve endings of primary sensory neurons. They are unlike more specialised sensory receptors for touch and pressure. They are widely distributed and often coactivated. The initial pain perceived by a nociceptor is via $A\delta$ axons. More longer lasting duller pain is perceived by C fibres. Receptors on the membrane of the nociceptor axon convert stimuli into a depolarising action potential.

Nociceptors can be thermal, mechanical, polymodal and silent.

- Thermal nociceptors – usually activated by temperatures $>45^{\circ}\text{C}$ or less than 5°C . They are small diameter, thinly myelinated $A\delta$ axons (speed 5 to 30m/s).
- Mechanical nociceptors – activated by more intense pressure (not light touch) and are also thinly myelinated $A\delta$ axons.
- Polymodal nociceptors – are activated by chemical, thermal and high intensity mechanical stimuli. They are small diameter unmyelinated C axon endings conducting at slower speeds of 1m/s.
- Silent nociceptors – these are found in viscera, and they are not normally activated by noxious stimuli. Inflammation and chemical stimuli reduce their firing threshold, and they contribute to secondary hyperalgesia and central sensitization.

The transient receptor potential vanilloid receptor family, and in particular, transient receptor potential vanilloid receptor 1 (TRPV1) is expressed in oesophageal epithelial cells. TRPV1 is activated by weak acid as well as capsaicin and thermal stimuli (24).

Weak acid is also known to activate the acid-sensing ion channels (ASICs), which belong to the voltage-insensitive, amiloride-sensitive degenerin/epithelial Na channel superfamily (25). ASIC3 in particular, is known to be expressed on human oesophageal epithelial cells (26).

Another important receptor is Protease-activated receptor-2 (PAR-2), also expressed in human oesophageal epithelial cells. It is thought to increase acid-induced Adenosine triphosphate (ATP) release. ATP behaves as a neurotransmitter in the central and peripheral nervous systems and is involved in peripheral inflammation and transmission of pain (27), by inducing the secretion of platelet-activating factor (PAF), IL-8, eotaxins, monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-1 (MIP-1) that contribute to inflammation and injury of the oesophageal mucosa. ATP can also mediate the release of other neurotransmitters such as substance P and CGRP that may contribute to transmission of the sensation of pain (28).

Wu et al demonstrated that acid induced ATP release was significantly reduced after pretreatment of human oesophageal epithelial cells with both 5-iodoresiniferatoxin (IRTX), a TRPV1-specific antagonist, and with amiloride, a nonselective ASIC blocker. They also showed that pretreatment of human oesophageal epithelial cells with a PAR-2 agonist enhanced weak acid-induced ATP release (29). These findings suggested that the pathophysiology of oesophageal pain hypersensitivity may be associated with the activation of PAR-2, TRPV1, and ASICs.

Prostaglandins are inflammatory mediators associated with inflammation and nociception, and of the prostaglandins, prostaglandin E2 (PGE2) is known to be associated with nociception. When stimulated with chemical, thermal, mechanical or inflammatory stimuli, levels increase, causing peripheral sensitization of adjacent nerve endings via nociceptors such as TRPV1 (30). Prostaglandins bind to EP receptors, and PGE2 in particular, binds to 4 subtypes of EP receptors -

EP1, EP2, EP3 and EP4. Of these subtypes, EP1 receptors have been shown to be associated with pain processing. Oesophageal acid exposure has been shown to increase PGE2 levels (31), whilst antagonists of EP1 such as ZD6416 (32) and ONO 8539 (33) have been shown to reduce oesophageal hyperalgesia.

There are other receptors and ion channels expressed in nociceptive sensory endings, although not specifically identified yet in the oesophagus. Those expressing tetrodotoxin resistant Na channels (such as $Na_v1.7$, also called SCN9A) were discovered in patients with a deletion causing the inactivation of $nav1.7$, resulting in a complete inability to sense pain (34). All other sensations are normal in these individuals. Efforts are ongoing to assess the viability of NAV 1.7 as a target for pain modulating therapies.

MrgX2, is a member of the Mas-related gene (Mrgs) family (which are a recently identified G protein-coupled receptor gene family) (35). MrgX2 is specifically expressed in nociceptive neurons of the dorsal root ganglia and trigeminal ganglia, suggesting a role in pain processing, with potential as another target for pain modulating therapies.

Normal oesophageal physiology

The primary function of the oesophagus is to convey ingested contents from the mouth to the stomach. This is achieved by a coordinated sequence of neuromuscular activity. Deglutition involves relaxation of the upper oesophageal sphincter, followed shortly by relaxation of the lower oesophageal sphincter, with a wave of peristaltic contraction propagating from proximal to distal oesophagus, thus allowing the bolus to be admitted to the stomach.

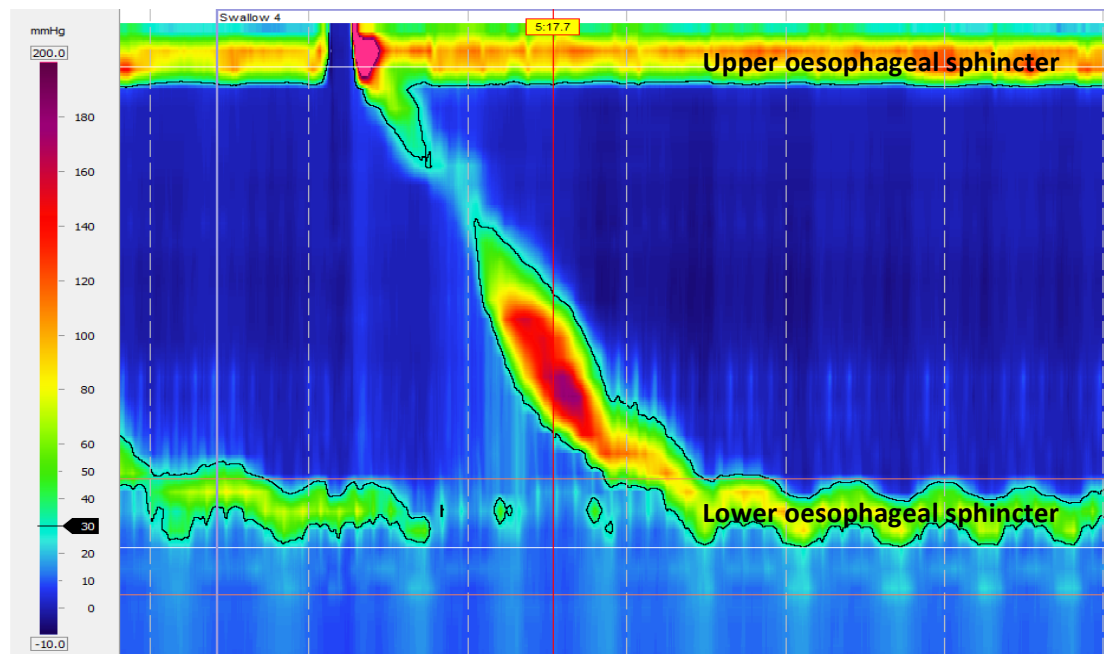


Figure 1.2: High resolution manometry showing the high pressure zones of the upper oesophageal sphincter (UOS) and the LOS. The colours on the heat map represent pressure, the oesophagus is represented on the y axis and time is represented on the x axis.

A physiological mechanism for venting gas from the stomach exists, and involves prolonged relaxations of the lower oesophageal sphincter, which occur independently to swallowing (36). These relaxations are termed transient lower oesophageal sphincter relaxations (TLOSRS) and are stimulated by gastric distension, especially of the proximal stomach.

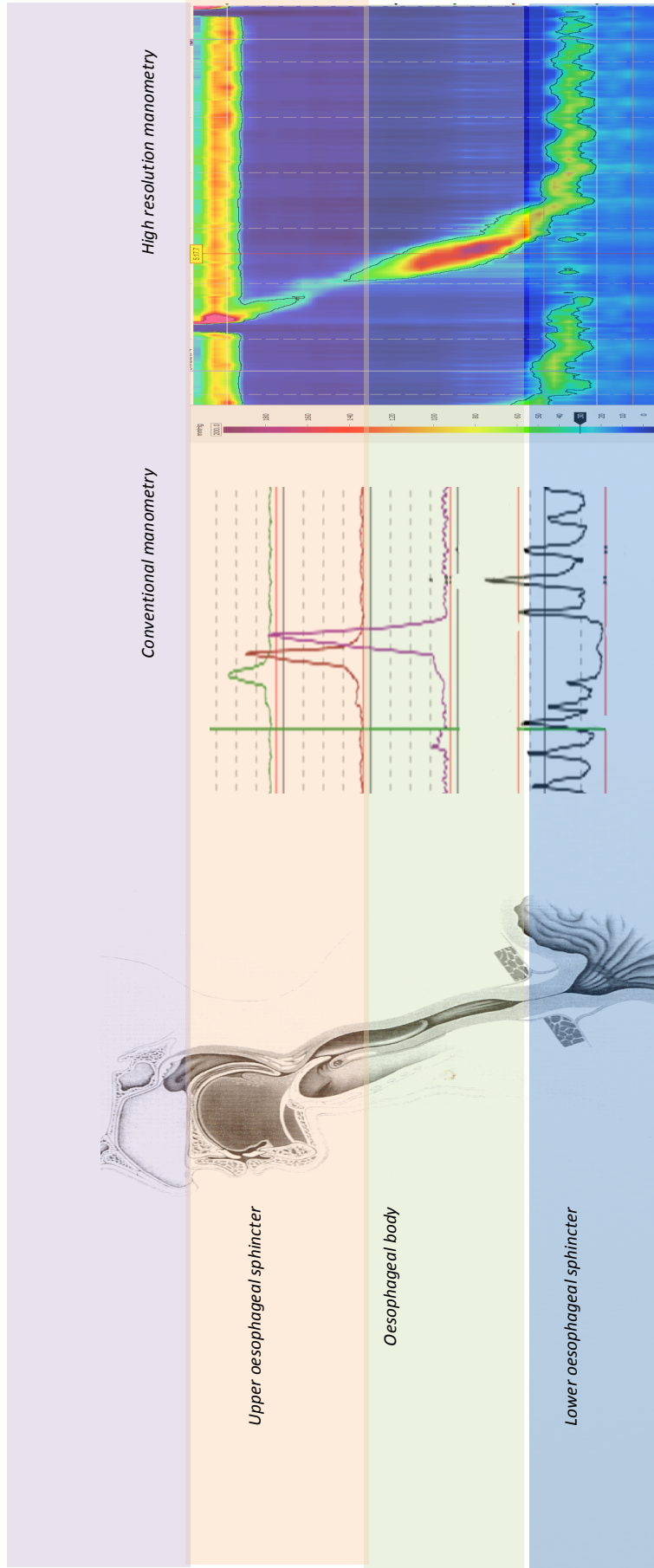


Figure 1.3: A representation of conventional water perfused manometry versus high resolution manometry, highlighting revolutionary improvement in visualisation of the UOS and LOS in particular.

Gastro oesophageal reflux disease

Gastro oesophageal reflux disease (GORD) is defined by the Montreal definition as a condition which develops when the reflux of gastric content causes troublesome symptoms or complications (1). GORD is then further classified by the Montreal classification system (Figure 1.4) into categories defined by investigations that form the usual diagnostic algorithm (Figure 1.6) for a patient with suspected GORD.

Patients with no evidence of oesophageal injury are deemed to have a symptomatic syndrome. Symptoms may be typical or atypical, but in general, patients with the symptomatic syndrome of GORD are defined as having non-erosive reflux disease (NERD).

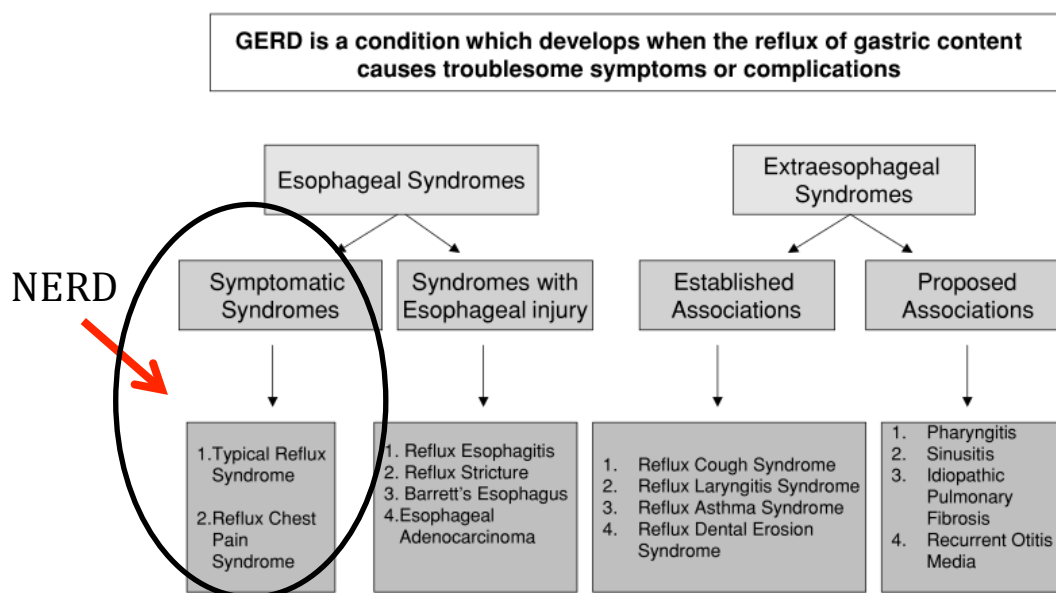


Figure 1.4: Overall definition of GERD and its constituent syndromes – Montreal classification.

Epidemiology

The reported prevalence of GORD is increasing worldwide, with rates of up to almost 30% in North America, almost 26% in Europe, 9% in East Asia, 33% in the Middle East, almost 12% in Australia and 23% in South America (37). In contrast, a similar analysis undertaken in 2005 reported much lower rates overall. For example, as a whole, in the Western world, prevalence rates were previously approximately 20% (38). The cause of this overall increase in prevalence is not easy to elucidate. The high economic burden of GORD is therefore only likely to rise. Peery et al reported that gastro oesophageal reflux was the most common GI diagnosis made in the United States in their 2012 update (39), with cost implications due to investigations (endoscopy) and treatment. In the UK, direct and indirect care costs of reflux disease are estimated to be in the order of £2 billion *per year* (40).

Non erosive reflux disease (NERD)

Of the large population of patients with GORD, those diagnosed with NERD make up 70% (41). This group of patients is a heterogeneous one and definition of the constituent component groups is ongoing.

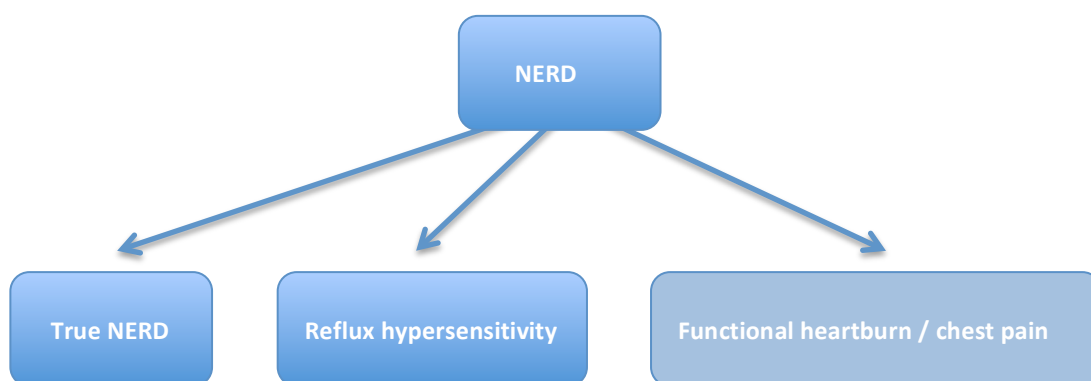


Figure 1.5: Patients with functional heartburn are often mistakenly considered as NERD until this is disproven with 24 hour multi channel intra luminal impedance (MII) pH studies.

As shown above, those patients with oesophageal reflux hypersensitivity and patients with functional heartburn are also included as NERD until 24 hour *multi channel intra luminal impedance* (MII) pH studies are carried out. The significance of this is shown when response rates to PPI therapy are reviewed, when patient with FH or FCP do not respond to anti reflux therapy

True NERD

These are patients who have no macroscopic oesophageal injury at OGD and who have raised oesophageal acid exposure on 24 hour pH metry alone. The proportion of these patients is not as high as previously anticipated with the advent and wider use of MII pH metry (42).

Oesophageal reflux hypersensitivity

These are patients who have no macroscopic oesophageal injury at OGD, and who have normal oesophageal acid exposure but positive reflux-symptom association at MII pH metry.

Functional heartburn and functional chest pain

These are patients who have no oesophageal injury at OGD and who have normal oesophageal acid exposure as well as negative reflux-symptom association at MII pH metry (4).

Diagnostic tools in GORD

On presenting to a clinician, a patient with symptoms of GORD currently will either be offered a trial of proton pump inhibitors (PPIs) or be referred for an oesophago-gastro-duodenoscopy (OGD). As mentioned above, most OGD examinations yield a negative finding - that is they show a macroscopically normal upper gastrointestinal tract. Oesophageal biopsies to look for possible histological evidence of eosinophilic oesophagitis and microscopic oesophagitis can be performed through the endoscope at the same time.

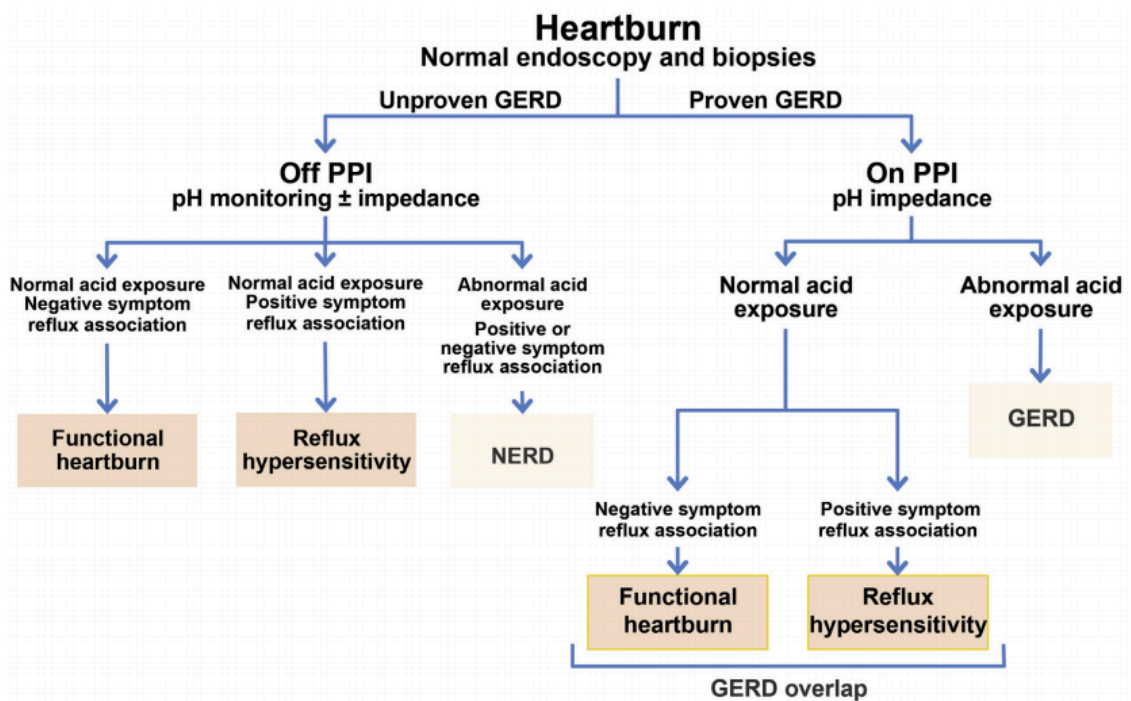


Figure 1.6: Classification of heartburn in the context of a normal gastroscopy as per Aziz et al from the ROME IV oesophageal disorders section, highlighting the use of pH monitoring in classification (2).

Prior to pH metry, patients undergo high-resolution manometry (HRM), in order to exclude major motility disorders, as well as to locate the upper border of the LOS. The next step in investigating these patients is by means of 24 hour pH monitoring, either using an oesophageal

catheter (with or without impedance recording) or wirelessly with a pH capsule attached to the distal oesophagus.

Recently, the length of pH studies has come under scrutiny. Sweis et al (43) took 38 patients who had a negative 24 hour catheter based pH study and who continued to have symptoms. They then performed prolonged wireless pH studies (up to 96 hours, with a median of 72 hours) on these patients. Average versus worst day analysis demonstrated that oesophageal acid exposure was 37% versus 47%. More strikingly, when symptom association probability (SAP) was calculated, average versus worst day analysis showed a positive SAP in 34% versus 63% respectively. Overall, using average and worst day analyses, 61% and 76% of these patients were diagnosed with GERD. This not only highlights the increased tolerability of wireless pH studies, but also considering a negative SAP would confer a diagnosis of functional heartburn or chest pain, the concern is that these patients may be erroneously diagnosed as having functional heartburn / chest pain, and not GORD, limiting their access to potentially helpful treatments.

Another area of debate is the reliability of calculating symptom association using either Symptom Index (SI), symptom specificity index (SSI) or SAP. SI and SAP are more commonly used. All three are a means of reconciling the probability of a reflux event and an episode of heartburn and are therefore used to assess reflux symptom association. All three statistical methods of calculating symptom association are more useful when patients experience higher rates of reflux. Slaughter et al concluded that SI and SAP were less reliable in those patients with low numbers of reflux events (less than 10% of the physiologically accepted number of reflux episodes), and SI and SAP were also less reliable in those who responded poorly to PPI therapy (44), with the effect of overestimating symptom association. Barriga-Rivera et al used the Monte Carlo method to evaluate reflux symptom association, and concluded in particular that the length of the study did not impact on SI and SSI, and in fact, the longer the study, the more reliable they were. However, in terms of SAP, the longer the study duration, the higher the value of the SAP was due to a non linear relationship between SAP and duration. As discussed above, length of pH studies is a topic of review recently, and with the

advent of longer duration studies, the utility of the SAP will increasingly come under scrutiny (45). Therefore, it is important to note these limitations of the current available methods of calculating reflux symptom association when interpreting results.

The Bernstein test was developed to diagnose reflux induced chest pain (46). It is still occasionally used to diagnose an acid sensitive oesophagus in the clinical setting, although currently a modified version is often used in the research setting to look at changes in the acid sensitivity of the oesophagus in relation to various therapies. During this test, saline is initially infused into the lower oesophagus (10 cm above the lower oesophageal sphincter) at a rate of 10 mL/min for 2 minutes. Subsequently, without the patient's knowledge, 0.1 M hydrochloric acid solution is infused into the lower oesophagus for 10 minutes at the same rate.

During a modified Bernstein test, stimulus-response functions to acid are quantified by lag time to symptom perception (the time taken for subjects to report when retrosternal discomfort is perceived during acid perfusion). In the context of research, sensory intensity rating (at the end of the acid perfusion) and APSS (acid perfusion sensitivity score) are often calculated (47).

Treatment of GORD

<i>PPIs</i>	<i>Omeprazole, lansoprazole, rabeprazole, pantoprazole</i> <i>Esomeprazole</i> <i>Dexlansoprazole modified release</i>
<i>PPI combinations</i>	<i>IR omeprazole (combined with sodium bicarbonate)</i>
<i>H2 Receptor antagonist</i>	<i>Ranitidine</i>
<i>Motility agents</i>	<i>Domperidone, metoclopramide, erythromycin</i>
<i>Visceral hypersensitivity</i>	<i>Citalopram</i>
<i>Surgical therapies</i>	<i>Nissen Fundoplication</i> <i>Endostim device</i> <i>Linx device</i>
<i>Endoscopic therapies</i>	<i>Stretta procedure</i> <i>Endocinch system</i> <i>Esophyx device</i> <i>MUSE™ system</i>

Table 1.3: Treatments either currently available or, in development, for the treatment of GORD.

Acid suppression therapy

Proton pump inhibitors (PPIs) are currently the most commonly used pharmacological therapy used for the treatment of GORD. The most widely used PPIs are enteric coated, to delay release of the inactive drug until it is in the small bowel. Once in the small bowel, the inactive PPI is quickly absorbed and redistributed to the acid filled canaliculus of an actively secreting gastric parietal cell. Here, in the acidic environment of the canaliculus, it is activated. The active PPI binds to a cysteine moiety in the proton pump, irreversibly stopping acid secretion (48). Newer isomers of traditional PPIs such as esomeprazole (49-51), dexlanzoprazole (52, 53) and S-pantoprazole have an increased bioavailability profile, with an increased area under the plasma concentration–time curve. Extended release preparations have been developed to increase exposure of more proton pumps to the PPI. Extended release rabeprazole contains a combination of a standard enteric coated tablet

which is released in the proximal small intestine, and four pulsatile release tablets that are released in the distal small intestine and the colon (54). Alevium (AGN201904-Z) is a chemically metered absorption omeprazole that is slowly absorbed throughout the length of the small intestine resulting in a steady and prolonged plasma residence time (55). Immediate release (IR) PPIs are another avenue that have been explored. IR omeprazole and IR esomeprazole are non-enteric coated and combined with (54) sodium bicarbonate. The sodium bicarbonate prevents the PPI from being activated in the stomach as it passes into the small intestine. Because it does not have an enteric coat, the PPI is more rapidly absorbed. The sodium bicarbonate also stimulates acid secretion, enhancing uptake and activation of the inactive PPI (56). PPIs have been combined with other agents to try to enhance their acid suppressive action. VECAM is omeprazole combined with VB101, a compound with pentagastrin like activity, which activates the parietal cell and stimulates acid secretion from the proton pump. This allows more PPI to be taken up in to the parietal cell canaliculi, enhancing PPI activity, thus reducing reliance on meal times as the proton pump is independently activated (56). PPIs have been combined with nitric oxide, which is known to have an effect on mucosal blood flow and mucous production, with H2RAs, alginates and with prokinetics (56). Other newer acid suppressants under development include tenatoprazole (an imidazopyridine with a longer half life than traditional PPIs) (57) and Ilaprazole (a modified benzimidazole that may be useful in poor PPI metabolizers as it is metabolized via a different pathway than the usual CYP2C19 pathway) (58).

Potassium competitive acid blockers (PCABs) bind to the potassium-binding region of the proton pump in a competitive reversible manner (59). The onset of action is also much faster than in traditional PPIs. Of the PCABs recently under development, TAK 438 (Vonoprazan) is one that has recently completed Phase 3 trials at present. Although previous studies demonstrated noninferiority to lansoprazole in the treatment of erosive reflux disease (ERD) (60), at doses of 10 mg and 20 mg, Vonoprazan was not superior to placebo in terms of proportion of days without heartburn in

patients with NERD. Severity of heartburn however was lower with vonoprazan compared to placebo (61).

Anti-reflux agents

Although much progress has been made with acid suppression, it is clear that this is not likely to address all symptoms in GORD, particularly regurgitation and symptoms associated with non-acid reflux. Reducing reflux per se is a more direct approach and is currently achieved by surgical fundoplication. Pharmacological options to reduce reflux do so by reducing transient lower oesophageal sphincter relaxations (TLOSRS). TLOSRS are a major mechanism of reflux in healthy subjects and in patients with GORD (62, 63). They are prolonged relaxations of the lower oesophageal sphincter that occur independently to swallowing and are a means by which gas from the stomach is vented (36). They occur more in the upright position compared to the supine position (64), and they are more likely to be associated with acid reflux in patients with GORD (65). TLOSRS are stimulated by gastric distension, especially of the proximal stomach (36). This distension triggers vagal afferents that synapse in the NTS, which then activates motor neurons in the dorsal motor nucleus of the vagus nerve, leading to relaxation of the lower oesophageal sphincter. During a TLOSRS, there is also associated crural diaphragmatic inhibition, contraction of the costal diaphragm and prolonged oesophageal shortening due to longitudinal muscle contraction in the distal oesophagus (66), and these all contribute to the occurrence of reflux. Given that TLOSRS are a major cause of GORD, they are an attractive therapeutic target, and several agents have been developed in an attempt to reduce their frequency. The GABA(β) agonist, baclofen, is able to reduce TLOSRS frequency and number of reflux events in patients with GORD (67). Unfortunately baclofen crosses the blood brain barrier, and the subsequent central side effects (such as drowsiness) have prohibitively restricted its use in clinical practice. This has led to the search for better-tolerated TLOSRS inhibitors. Recently, the metabotropic glutamate receptor 5 (mGluR5) antagonist AZD2066 was demonstrated to reduce TLOSRS and reflux episodes in healthy male volunteers in a dose dependant fashion (68), but a study in patients with NERD was terminated early due to safety issues

(ClinicalTrials.gov Identifier: NCT00939094). Previous mGluR5 antagonists had issues with poor tolerability and hepatotoxicity.

Recent attempts at development of nitric oxide synthase inhibitors and cannabinoid agonists as TLOSR inhibitors have been halted due to issues with tolerability. GABA(β) agonists with improved side effect profiles are perhaps the most promising candidates for TLOSR inhibition in clinical practice. Lesogaberan and AZD9343, which are both peripherally acting GABA(β) agonists have been shown to reduce TLOSR frequency and acid reflux as well as increase LOS pressure (69-71). Lesogaberan particularly was shown to reduce episodes of reflux more than it reduced TLOSRS, suggesting that the effect of Lesogaberan on other mechanisms associated with reflux such as LOS pressure reduction are important as well. Lesogaberan also appeared to have a greater effect on acid reflux events compared to non-acid or weakly-acid reflux events. This effect has also been seen with Baclofen. The cause of this was unclear, and although the use of Lesogaberan in acid reflux appeared promising, its potential use in oesophageal hypersensitivity in relation to non-acid and weakly-acid reflux could also have been considered (71). Arbaclofen Placarbil, which is an actively transported prodrug of the active R-isomer of the GABA(β) agonist baclofen was also shown to reduce reflux episodes compared to placebo in initial studies (72). Such drugs may also be potential treatments in reducing belching related persistent reflux, due to their action on increasing LOS pressure. Lesogaberan and Arbaclofen Placarbil were both taken further in larger studies (73, 74). Unfortunately, although they do reduce TLOSR frequency, increase LOS pressure and reduce reflux episodes, clinical efficacy was limited in trials, and both have now been abandoned by their respective pharmaceutical companies (71, 72, 75). As a result, at present, baclofen remains the only clinically available TLOSR-inhibiting agent for patients with refractory disease. It is possible that patient selection was a significant factor in failure of these drugs in the clinical setting, but adverse events appeared to have played a role as well. In these trials, patients were not previously phenotyped based on reflux monitoring and so functional heartburn or functional dyspepsia could not be excluded from the studies. This has implications on the outcome of such trials because, in

these patients, reducing TLOSRS may not have been expected to improve their symptoms. More care in phenotyping and selection of patients for trials of TLOSRS inhibitor therapy may yet determine a role for these drugs in GORD management.

Alginates and prokinetic agents

The importance of the acid pocket (the a layer of unbuffered acid that sits on top of gastric contents in the postprandial period) in GORD has been increasingly recognized over recent years (76, 77). Modulation of the position or composition of the acid pocket is an interesting therapeutic modality. Alginate preparations can form a raft on top of gastric contents, and have been shown to be able to neutralize and displace the acid pocket in patients (78). It is possible that further exploitation and modification of this effect may be useful in future therapies for GORD. Alginates may also have a secondary beneficial effect in protecting the oesophageal mucosa against noxious injury in reflux disease. This has been demonstrated *ex vivo* (79), and further exploration of this property may be of benefit in future drug development.

Macrolides such as erythromycin have been known to increase gastrointestinal motility, acting via motilin receptors. They also increase proximal stomach tone and LOS pressure (80). Recently, azithromycin was shown to reduce acid reflux events and oesophageal acid exposure in patients with GORD. Hiatus hernia in these patients with GORD was reduced in size and displaced more distally, moving the acid pocket more distal relative to the diaphragm. Newer motility agents such as mitemcinal (GM-611) have shown some promise but are still in the development phase (81).

Other prokinetic agents widely in use include domperidone and metoclopramide. Their utility is limited in the longer term, due to QT prolongation which is a well recognised phenomenon.

Mechanical treatments

Laparoscopic fundoplication techniques such as Nissen Fundoplication have become increasingly popular since the 1990s, with minimal-access approaches improving recovery times and comorbidity associated with fundoplication. Concerns about long-term morbidity, failure and side effect of laparoscopic surgery (particularly abdominal bloating, inability to belch, and dysphagia) (82) have resulted in attempts to develop even less invasive surgical procedures and devices.

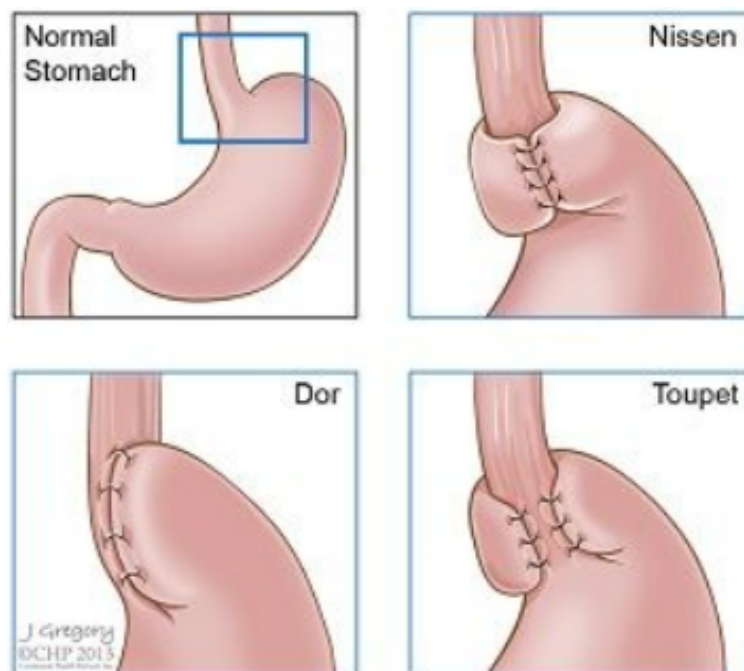


Figure 1.7: Types of laparoscopic fundoplication, all of which aim to increase integrity of the GOJ.

Another method in use is a laparoscopically placed magnetic ring (of interlinked titanium beads with magnetic cores connected by small wires) that is placed around the distal oesophagus.

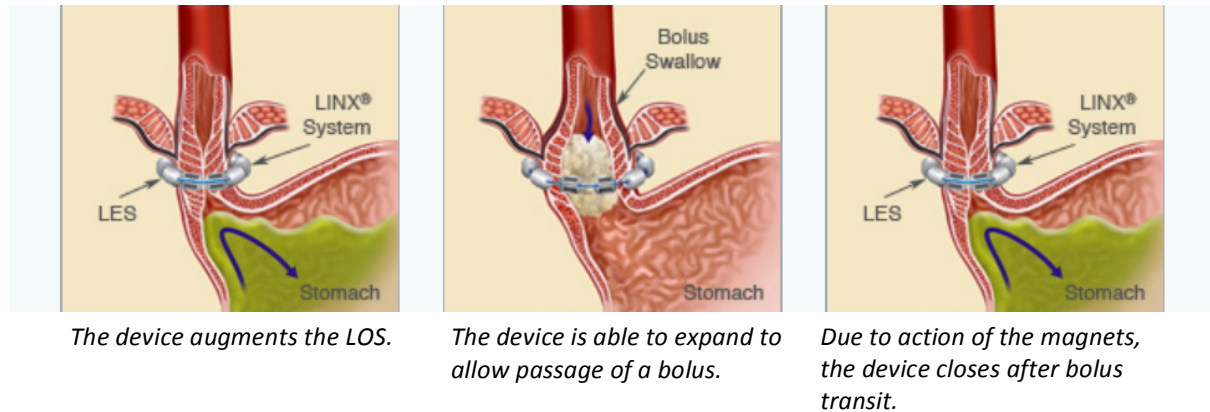


Figure 1.8: The LINX reflux management system (Torax Medical Inc, Minnesota)

<http://www.toraxmedical.com/linx/>

This device, the LINX reflux management system (Torax Medical Inc, Minnesota), essentially augments LOS pressure by constricting the LOS. Several studies have assessed efficacy in long term reduction of symptoms of GORD, reduction in PPI use and improvements in quality of life (83, 84).

Electrical stimulation

A newer mechanical method of treating GORD is an LOS stimulation device, the EndoStim (BV, The Hague, Netherlands). This was developed on the principle that gastro oesophageal reflux results from the failure of the barrier function of the GOJ, a major component of which is the LOS. Acute and chronic electrical stimulation of the LOS in animal models has shown increased LOS basal pressures (85) (86, 87). A short-term study in human subjects was published in 2012 by Rodriguez et al (88), where patients with GORD undergoing laparoscopic cholecystectomy, had a temporary electrode inserted into the LOS at the time of surgery. Oesophageal manometry was carried out before and during 30 min periods without LOS electrical stimulation, and this showed that stimulation increased resting LOS pressure, whilst peristaltic amplitude and residual LOS pressure (during swallowing) was not affected. Therefore, the GOJ is augmented, resulting in less reflux, and consequently reduced exposure of oesophageal mucosa to acid (as well as non acid) reflux. The same group further examined this technique with a 2 year open-label pilot trial of long-term LOS electrical stimulation with a permanently implanted stimulator in 24 patients with GORD (89), followed by a further year of follow up in a multicenter registry trial, showing significant and sustained improvement in distal oesophageal acid exposure, GORD symptoms and PPI use (90).

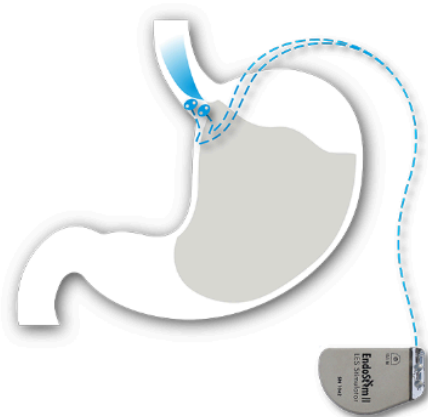


Figure 1.9: The Endostim device delivers mild electrical stimulation the LOS for pre-programmed periods, increasing LOS pressure without diminishing LOS relaxation.

Endoscopic therapies

Endoscopic anti-reflux therapies are gaining popularity as an even lesser invasive means of treating GORD mechanically. Stretta is an endoscopic thermal ablation technique where heat is applied to the lower oesophageal musculature via specialised needles attached to a catheter. Although GORD symptoms are reduced, distal oesophageal acid exposure is not, suggesting that the improvement is more likely due to thermal neurolysis of sensory nerves in the lower oesophagus (91).

Several trans oral incisionless fundoplication systems have been developed of recent, although with little success in terms of long term efficacy apart from the EsoPhyx device. A recent long-term efficacy study assessing the an updated version of the Esophyx device over a 6 year period, showed reduced PPI use, improved symptom scores and reduced reflux episodes (92).

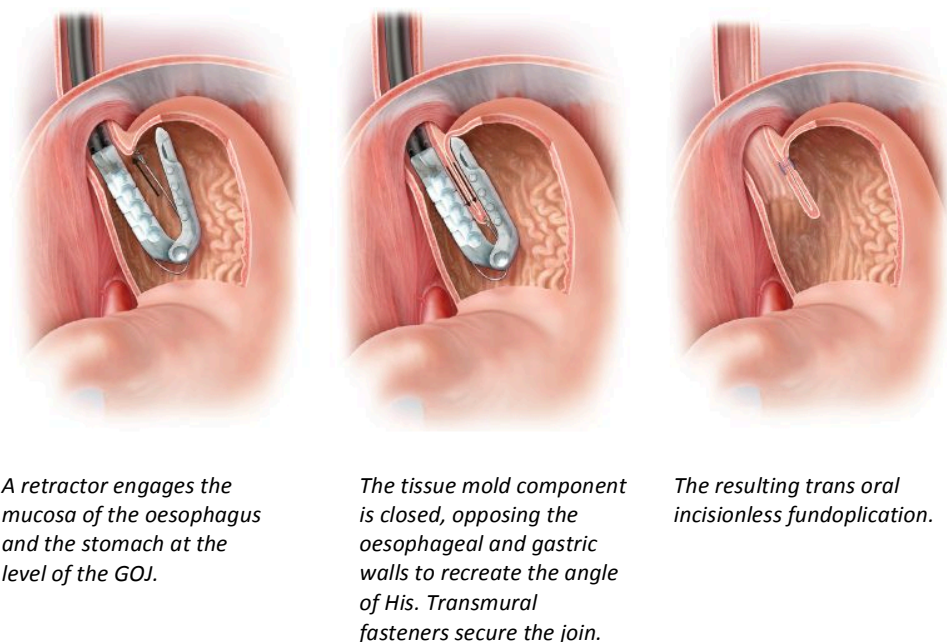


Figure 1.10: Transoral incisionless fundoplication with the EsoPhyx device (93).

Treatment of oesophageal hypersensitivity

In reflux disease there may be a heightened perception to the gastro oesophageal refluxate, and this may be via peripheral, central or psychoneuroimmune sensitization. Visceral hypersensitivity is increasingly being considered as an important aspect of GORD pathophysiology, particularly in non-erosive disease (94). Subsequently it is increasingly seen as a therapeutic target for treatment of GORD. Peripheral sensitization is where direct noxious stimuli such as acid cause the activation of nociceptive channels leading to pain hypersensitivity at the site of injury (primary hyperalgesia). This can be mediated via direct or indirect activation of local nociceptive receptors, and in the oesophagus, the acid-sensitive TRPV1 receptor is a strong candidate for therapeutic targeting (28). As well as chemical and thermal activation, the TRPV1 receptor can also be activated by even mild acidification (95), and therefore an antagonist may be useful in visceral hypersensitivity. TRPV1 antagonists have been keenly sought (96-98). AZD1386 is a TRPV1 antagonist that increased oesophageal pain thresholds to heat in healthy volunteers (99). Unfortunately, it has now been shown to have no analgesic effect on oesophageal pain thresholds in NERD patients with partial PPI response (100).

Central sensitization occurs with repetitive stimulation of peripheral nociceptors, leading to hypersensitivity in areas remote from the area of peripheral sensitization (101). This is called secondary hyperalgesia (102). Increased visceral hypersensitivity has been demonstrated in the upper oesophagus of patients with GORD when the lower oesophagus was stimulated with acid. Similarly, proximal oesophageal and chest wall hyperalgesia has been demonstrated in healthy volunteers following distal oesophageal acidification (103, 104). This secondary hyperalgesia is attenuated by prostaglandin E2 receptor-1 (EP-1) antagonism (105), as was shown with the EP-1 antagonist ZD6416. Further development of this was halted due to adverse events. A newer EP-1 receptor antagonist, ONO 8539 has been developed. A Phase IIb clinical trial evaluating ONO 8539 is the subject of Chapter 3 in this thesis.

Targeting central sensitization remains an important area for future drug development in patients with reflux symptoms, since central sensitization may play an important role in not only NERD, but also functional heartburn.

Few other drugs have been evaluated in reflux hypersensitivity. Viazis et al looked at citalopram as a treatment RH, and demonstrated that citalopram reduced heartburn symptoms significantly compared to placebo ($P = 0.021$) (106). Prior to this, Broekaert et al demonstrated that in the acute setting, Citalopram 20mg administered intravenously significantly increased sensitivity and discomfort threshold during balloon distention of the oesophagus. Citalopram also significantly increased lag time to perception of heartburn during distal oesophageal acid perfusion (107).

Overlap between GERD, NERD, RH and FH

With the introduction of ROME IV, the understanding that there is likely an overlap between GORD, NERD, RH and functional oesophageal disorders is an important consideration in the evaluation of therapies. Therefore, considering that a patient with ERD may have an element of oesophageal hypersensitivity, if they are refractory to anti reflux and acid suppression therapies, this increases the therapeutic options available for this patient for the treatment of their refractory symptoms.

Treatments for functional oesophageal disorders, such as functional chest pain and functional heartburn are summarised in table 1.3. Most have been evaluated for use in non cardiac chest pain, which is used as an umbrella term for chest pain of oesophageal origin. Although functional chest pain is considered to fall within this category, GORD as a cause of non cardiac chest pain is not ruled out. The varied response rates of these treatments may add weight to the idea that diagnoses causing symptoms of heartburn and chest pain may lie on a spectrum ranging from ERD to FH and FCP.

Class of drug	Dose	Disorder	RCT	Side effects	Response
TCAs					
Imipramine	50 mg/day	NCCP	+	+/-	57%
Amitriptyline	10–20 mg/day	NCCP, globus	+	+/-	52%
SSRIs					
Sertraline	50–200 mg/day	NCCP	+	+	57%
Paroxetine	50–75 mg/day	NCCP	+	+/-	Modest
Citalopram	20 mg/day	ES	+	+/-	Significant
Trazodone					
Vs clomipramine	50/25 mg/day	NCCP	-	+	Modest
Trazodone alone	100–150 mg/day	dysmotility	+	+/-	29%–41%
SNRIs					
Venlafaxine	75 mg/day	NCCP	+	++	52%
Other					
Theophylline	200 mg twice/day	NCCP	+	+/-	58%
Gabapentin	300 mg 3 times/day	globus	+	+/-	66%

Table 1.4: Pain modulating therapies evaluated for the treatment of functional oesophageal disorders (2).

Other therapies evaluated in the treatment of functional oesophageal disorders include Imipramine (108) and Amitriptyline (109) which are both tricyclic antidepressants (TCAs), and have demonstrated benefit in terms of symptoms over placebo and Rabeprazole respectively. As mentioned above, the SSRI Citalopram has been shown to confer some benefit in RH (106, 110). Similarly, Paroxetine (110) and Sertraline (111) have been evaluated in non cardiac chest pain as potential therapies. Trazodone (112, 113) and Venlafaxine (a selective norepinephrine reuptake inhibitor (SNRI)) have both been shown to modestly improve symptoms in non cardiac chest pain. Theophylline has been evaluated in non cardiac chest pain, showing improvement in symptom scores (114). This work was based on studies showing that patients who were pretreated with intravenous aminophylline (an antagonist of adenosine P1-receptors) showed reduced adenosine induced chest pain compared to placebo (115).

The oesophageal pain hypersensitivity model

Our group has developed a model in which infusion of 0.15M hydrochloric acid into the healthy distal oesophagus (figure 1.11 and figure 1.12) reduced pain threshold not only in the acid exposed region (due to peripheral sensitization of afferent nerves), but also in the adjacent unexposed region due to central sensitization of spinal dorsal horn neurones (103) (figure 1.12).

Evidence of the facilitation of afferent sensory pathways in the model has been obtained by a cortical evoked potential study demonstrating a decrease in latency and increase in amplitude of the response after acid infusion in comparison to saline (116). The factors that mediate post-acidification oesophageal sensitization are incompletely understood but physiological factors are likely to be involved.

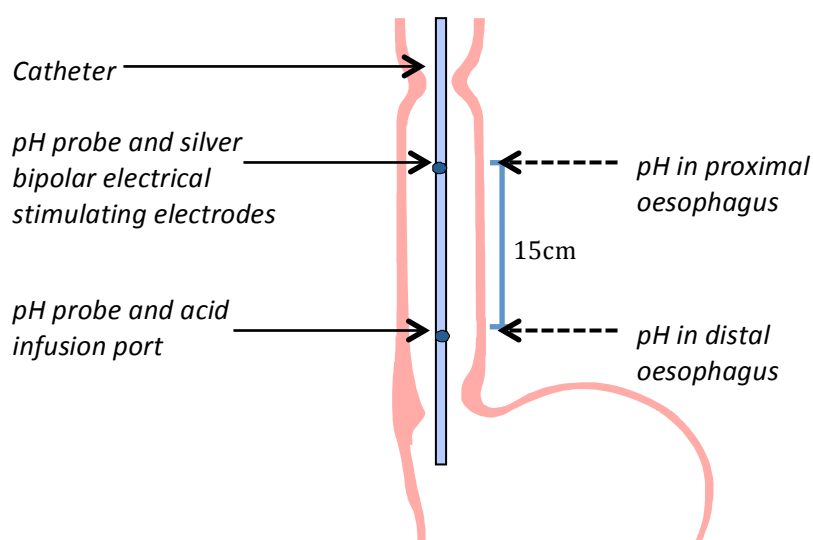


Figure 1.11: A catheter is placed in the oesophagus, which has a proximal pH probe & silver bipolar electrical stimulation electrodes to measure oesophageal pain sensitivity and a distal pH probe & infusion port (12).

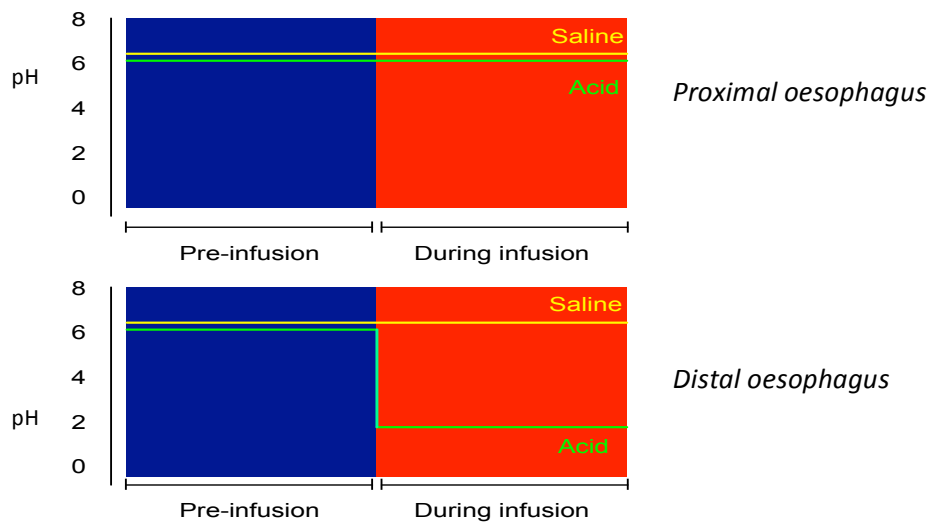


Figure 1.12: Subjects are randomised to receive either a saline or acid infusion. As expected when saline is infused there is no change in pH in either the proximal or distal oesophagus, whereas there is a demonstrable drop in pH in the distal, but not in the proximal, oesophagus during acid infusion (12).

For instance, it has been demonstrated that oesophageal acidification is associated with a rise in sympathetic nervous system (SNS) activity and a fall in parasympathetic nervous system (PNS) activity, with subjects who withdrew their parasympathetic tone the most, developing the most heightened sensitivity (117) (figure 1.13).

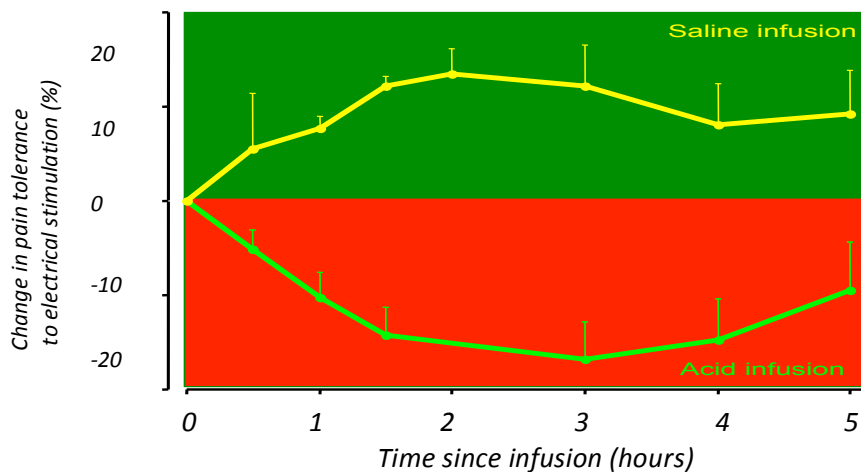


Figure 1.13: Pain thresholds in the proximal oesophagus, which has not been exposed to acid, show decreased pain sensitivity (shaded green area) due to habituation following saline infusion. Following acid infusion there is increased pain sensitivity (shaded red area) due to central sensitization (12).

ANS modulation of oesophageal pain

The autonomic nervous system (ANS) is thought to play a role modulating pain. This occurs via its interaction with the peripheral and central nervous system. It is thought that dysfunction of the ANS is likely to play a role in pain perception (118). An increasing body of evidence from animal studies has proposed the importance of the PNS, or as is more commonly referred to in the literature, “cholinergic tone”, as a critical mediator the inflammatory/anti-inflammatory balance (119).

The oesophageal pain hypersensitivity model, developed and validated by our group demonstrates that in the event of distal oesophageal acidification, the proximal oesophagus which has not been exposed directly to acid exhibits a lowered pain threshold due to central sensitisation as described above. Botha et al studied the modulation of the ANS and its effect on oesophageal pain hypersensitivity (12) in healthy volunteers. They demonstrated that parasympathetic tone was increased with slow deep breathing compared to unpaced sham breathing (figure 1.14). This had an

effect on central sensitization, increasing proximal oesophageal pain threshold after distal oesophageal acid exposure (figure 1.15).

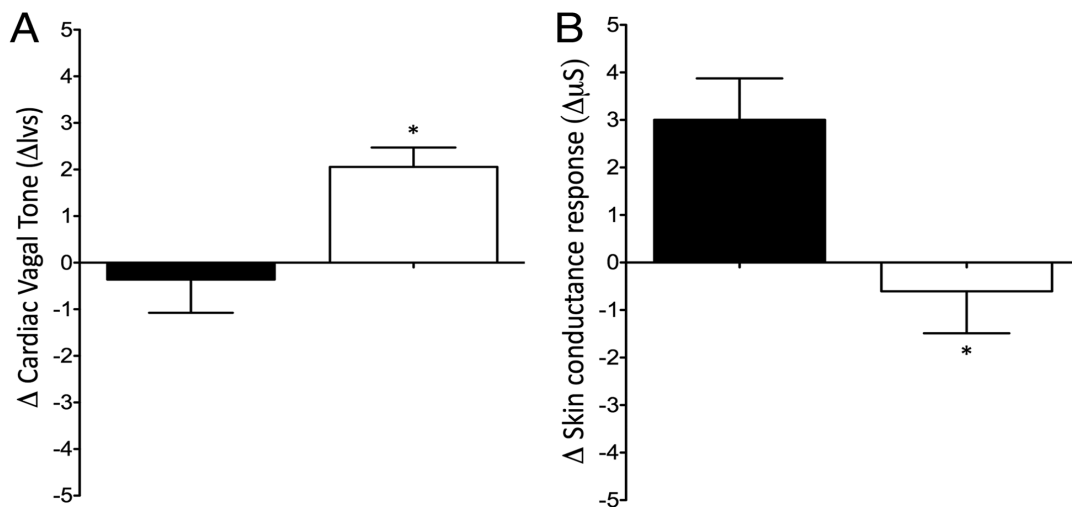


Figure 1.14: The effect of sham/un-paced breathing (shaded black) and deep breathing (unshaded) on cardiac vagal tone (mean \pm SE) (parasympathetic tone) and skin conductance response (mean \pm SE) (sympathetic tone). *Statistically significant at $p < 0.03$ (12).

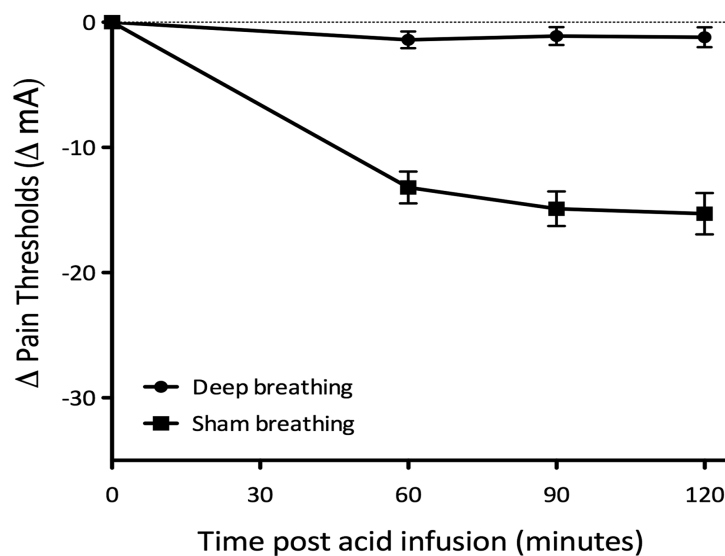


Figure 1.15: The effect of sham breathing (\blacksquare) and deep breathing (\bullet) on the development of central sensitisation, derived from the paired change in pain thresholds (mean \pm SE of the mean), in the proximal oesophagus at T60, T90 and T120, with mixed effects regression showing a coefficient of effect for deep breathing of 9.94 (CI 8.3 to 11.6, $p = 0.0001$) (12).

Botha et al clarified this further by using atropine, which is known to block parasympathetic tone. This was elegantly shown to abolish the central sensitizing blocking effect of deep breathing after distal oesophageal acid exposure (figure 1.16).

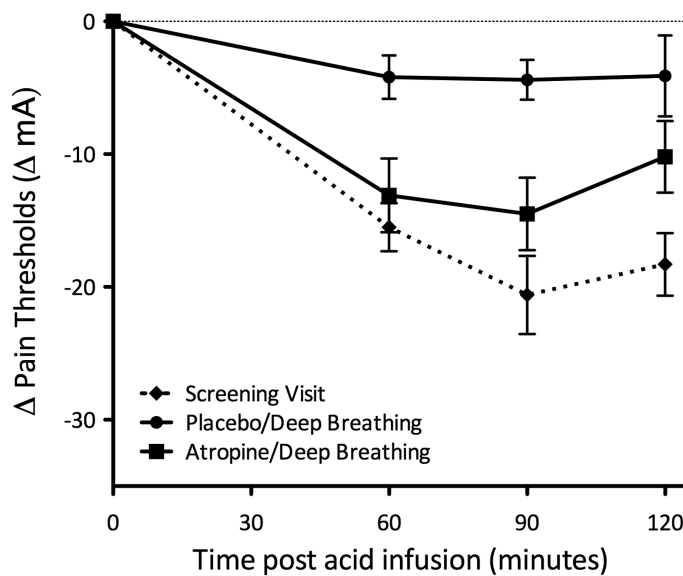


Figure 1.16: The effect of atropine/deep breathing (■) and placebo/deep breathing (●) on the development of central sensitisation, in comparison with the screening visit (◆), derived from the paired change in pain thresholds (mean±SE of the mean), in the proximal oesophagus at T60, T90 and T120. Mixed effects regression showed a significant effect for atropine (coefficient -3.5 mA/unit time (CI -6.8 to -0.06), $p=0.046$). (12)

The remaining questions addressed by this thesis

1. What characteristics “best” define patients diagnosed with oesophageal reflux hypersensitivity?

There is currently a paucity of data with regards to the characteristics of patients with a diagnosis of oesophageal reflux hypersensitivity. Although the definition of oesophageal reflux hypersensitivity is evolving, a better awareness of the characteristics of these patients will help in work to further understand the pathophysiology of this condition as well as inform efforts assessing potential therapies.

2. What is the prevalence and what are the characteristics of patients with oesophageal reflux hypersensitivity in our institution (Royal London Hospital)?

Current studies looking at the prevalence of oesophageal reflux hypersensitivity are limited. Looking at the prevalence and characteristics of our local population will not only address this issue, but also would be extremely useful in order to ensure better service provision for our local population.

3. Can we treat reflux hypersensitivity pharmacologically with newly developed drugs?
4. Can we treat reflux hypersensitivity non-pharmacologically by modulation of the autonomic nervous system (ANS)

There is a clear gap in therapeutics with regards to oesophageal pain that is fully or partially refractory to currently available therapy. Therefore, continuing the search for viable alternatives, be they pharmacological or non-pharmacological is a worthy exercise. Using our current understanding of the pathophysiology of oesophageal pain, and current advances in drug discovery as well as non invasive therapeutic techniques, further work is required to assess potential treatment strategies for oesophageal pain, and therefore oesophageal reflux hypersensitivity

Aim of this PhD research project

1. To review the current definitions and pathophysiology of oesophageal reflux hypersensitivity, and to assess the prevalence of oesophageal reflux hypersensitivity at the Royal London Hospital.
2. To assess pharmacological treatment of RH in patients with NERD using a selective prostaglandin EP1 receptor antagonist
3. To assess the effect of modulation of the ANS on oesophageal pain hypersensitivity in healthy subjects and patients with NERD using
 - a) transcutaneous vagal nerve stimulation
 - b) deep slow breathing

Chapter 2

Reflux hypersensitivity, definitions, pathophysiology and prevalence at the Royal London Hospital

Introduction

What is reflux hypersensitivity?

Oesophageal reflux hypersensitivity (RH) is defined as an increased sensitivity of the human oesophagus to mechanical, chemical and electrical stimuli in the absence of any macroscopic pathology or increased oesophageal acid exposure or reflux (2). This is not expected in the oesophagus of a healthy subject.

The concept of a hypersensitive oesophagus has been around since the 1990s with the advent of ambulatory oesophageal pH testing and reflux symptom association calculations. This was in response to the realisation that a population of patients suffering with typical GORD symptoms did so in the presence of physiological levels of distal oesophageal acid exposure. This group of patients with typical GORD symptoms that were related to physiological reflux was included as new category of non erosive reflux disease (NERD) in the ROME III criteria published in 2006 (120).

ROME is an international consensus effort to define, classify and foster research in functional gastrointestinal disorders (FGIDs). The ROME IV consensus document, released in 2016, updated diagnostic criteria for FGIDs.

In Rome IV in 2016 (2), Aziz et al reviewed the status of the hypersensitive oesophagus, placing it back in the realm of functional oesophageal disorders, defining it as oesophageal reflux hypersensitivity (RH). The rationale for this was due to the fact that the underlying pathophysiological process was thought to be more consistent with a functional disorder.

ROME IV diagnostic criteria for RH

It is stipulated that as RH is considered a chronic condition, the following diagnostic criteria must be fulfilled for the past 3 months with symptom onset at least 6 months before diagnosis with a frequency of at least twice a week.

1. Retrosternal symptoms including heartburn and chest pain.
2. Normal endoscopy and absence of evidence that eosinophilic oesophagitis (EoE) is the cause for symptoms.
3. Absence of major oesophageal motor disorders (achalasia/GOJ outflow obstruction, diffuse oesophageal spasm, jackhammer oesophagus, absent peristalsis).
4. Evidence of triggering of symptoms by reflux events despite normal acid exposure on pH or pH-impedance monitoring (response to antisecretory therapy does not exclude the diagnosis).

The pathophysiology of reflux hypersensitivity

Oesophageal biopsies of patients defined as having oesophageal hypersensitivity may have microscopic changes in the form of dilated intercellular spaces as well as microscopic oesophagitis. In patients with increased oesophageal acid exposure or increased reflux episodes but without macroscopic oesophageal injury (i.e. non-erosive reflux disease), microscopic oesophageal injury is seen in a majority of patients compared to controls as shown in a review by Dent et al (121). The predominant finding consistent with oesophageal injury was dilated intercellular spaces as well as basal cell hyperplasia and papillary elongation. Inflammatory cell infiltration was less consistent. Of interest, these changes responded to acid suppressive therapy (121). The lack of resolution of symptoms however is likely to relate to the longer lasting effects of peripheral and central sensitization in this instance. Therefore, although PPIs may address a component of the pathophysiology of RH,

part of the pathophysiological process remains untreated, hence the limited utility of PPIs in RH.

Although there is no direct evidence for neurogenic inflammation in RH, ROME IV suggests that abnormalities in transient receptor potential vanilloid 1 (TRPV1), acid-sensing ion channel 3, protease-activated receptor 2 (PAR2), neuropeptides such as substance P and CGRP, and their receptors such as neurokinin 1 receptor (NK1R) and receptor activity-modifying protein-1 may play a role in the pathophysiology of RH (26) (2).

In addition to this, ROME IV recognises that symptom perception in RH is likely to involve peripheral and also perhaps central sensitisation as well as other factors involved in the gut brain axis such as autonomic state, altered central processing of visceral stimuli and psychological factors, triggered by physiological reflux.

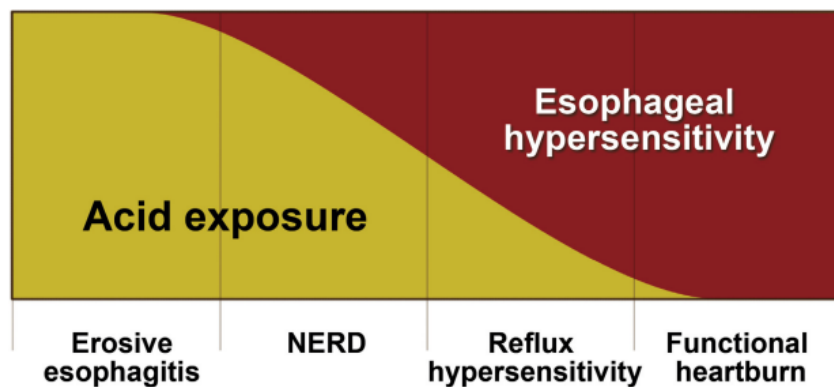


Figure 2.1: The interplay between esophageal hypersensitivity and acid exposure in the reflux symptom spectrum (2).

Diagnosis of reflux hypersensitivity

High resolution manometry

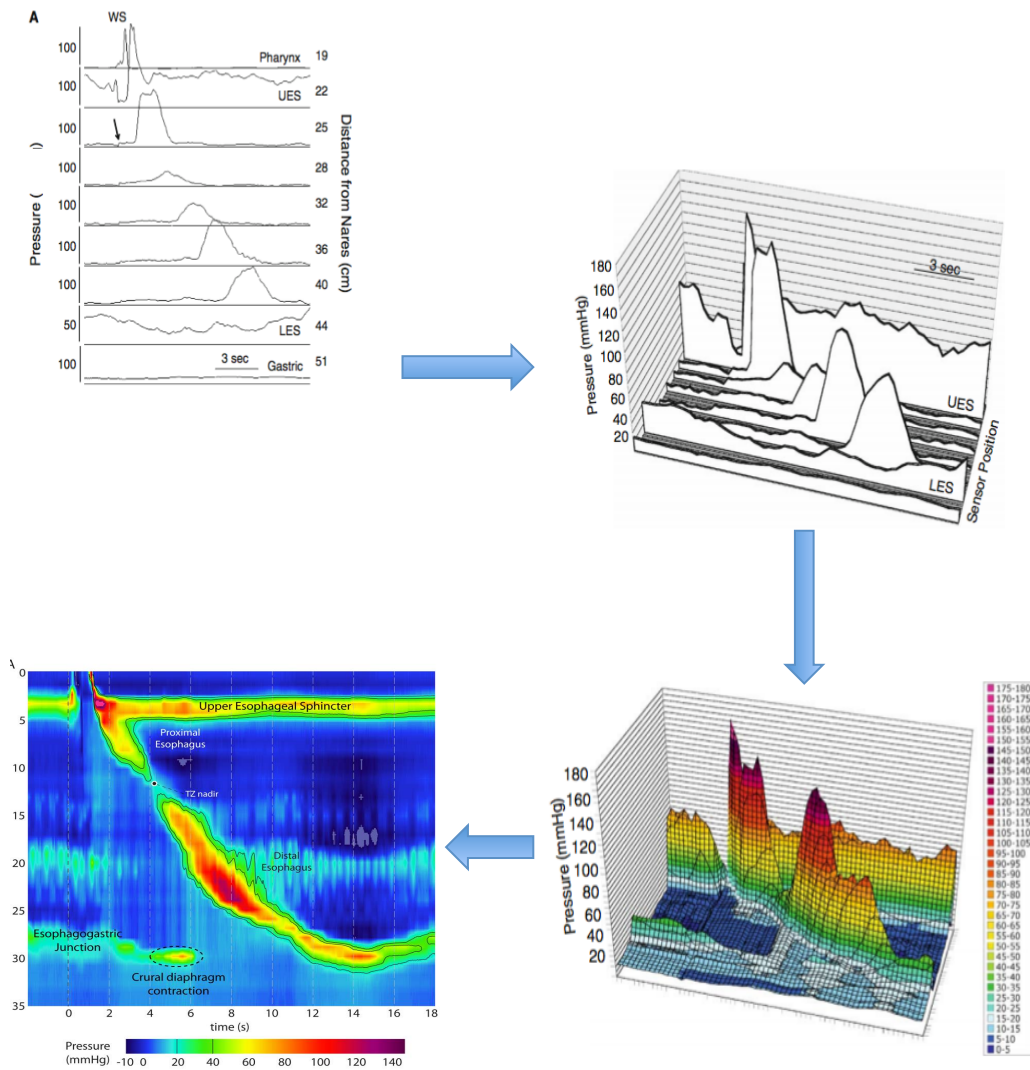


Figure 2.2 – This figure shows progression of a single Clouse plot with a pressure (at each position of the catheter) v time graph for each individual sensor to an aggregation of all 36 sensors on a pressure v time plot with sensor position on the z axis. Pressure is then divided into colour groups with high pressure reading as red and low pressure reading as blue. The y axis pressures are then coloured in with the respective corresponding pressure/colour groups. The 3 dimensional (pressure v time v catheter position) graph is then collapsed down to a 2 dimensional (time v catheter position) x v z axis graph with the y axis (pressure) represented by colour, akin to a topography map.

The current the gold standard test for assessing oesophageal motility is high resolution manometry (HRM) (122).

In the diagnostic algorithm described in chapter 1, HRM is performed prior to pH studies initially to exclude major oesophageal motility disorders as the cause of presenting symptoms. HRM is also useful to define the upper border of the LOS to ensure correct placement of the oesophageal and gastric pH sensors for accurate 24 hour pH studies.

The data acquired by the HRM catheter is analysed by ManoScan® software, and parameters are calculated using the latest Chicago Classification system version 3.0 (123) for oesophageal motility disorders. This then allows for the diagnosis of major and minor oesophageal motility disorders, which may have a bearing on the diagnosis of GORD, as well as helping elucidate possible causative or contributory factors such as hiatus hernia or poor oesophageal motility.

24 hour oesophageal pH and impedance testing

pH-metry is considered to be the gold standard investigation for the diagnosis of GORD (124). Currently, this may be performed with a catheter or a capsule based method. Adding impedance measurements allows for the quantification of non acid reflux and gas events as well as more detailed information on direction of travel of oesophageal contents. As mentioned previously, adding impedance measurements allows for the possibility of diagnosing GORD, and also RH, FH and FCP.

The 24 hour pH and MII pH testing system used at the Royal London Hospital GI physiology unit is a catheter based system. The MII pH system consist of a catheter with 2 antimony pH sensors (gastric and distal oesophageal) as well as 6 impedance sensing segments, each 2cm in length, sited at 3, 5, 7, 9, 15, and 17 cm above the proximal border of the LOS as defined by HRM. The catheter in both systems is single use. A compact flash

memory card is set up with the required testing details and inserted into the data logger box. The data logger box-catheter unit is then calibrated using pre prepared pH solutions.

Patients were instructed to keep a simple diary of meals and periods of being recumbent. Changes in position from upright to recumbent affect LOS pressure. Considering meal times, delayed gastric emptying for example has a bearing on gastric acid pH. A data logger carried by the patient allowed for the patient to record meals and periods of being recumbent.

In general, typical symptoms of reflux were assessed with regards to the assessing the possibility of association with reflux episodes. Atypical symptoms were occasionally assessed if they were particularly bothersome to a patient, and reflux was thought to be a possible cause. During a 24 hour pH or MII pH study, patients were given the opportunity to record upto three different symptoms. They were instructed to press a particular “symptom button” as soon as they started to experience a particular symptom. This data was logged in the data logger box carried by the patient for the duration of the recording as described previously.

The patient was able to go home after the above and continue with their usual activities of daily living, including going to work if feasible. They were asked to have meals and drinks as usual, but to avoid frequent snacking or ingesting acidic drinks. They were asked to continue to avoid acid suppression therapy, and to avoid antacids, alginates and other medications for reflux/GORD. The patient returned 24 hours later to have the catheter removed after which the data from the data logger was downloaded on to a secure network for analysis. The patient was debriefed and any questions or queries were addressed.

With pH metry, only acid reflux events and symptoms are apparent. With MII pH metry however, reflux events (liquid/gas/mixed) and their proximal extent, swallows, belches (gastric or supragastric) and their numbers are apparent as is the acidity of the refluxate (acid/non acid). The number and timing of symptoms, meal periods and recumbent periods are also apparent on the tracing.



Figure 2.3: 24 hour pH metry/ MII pH metry catheter and data logger (ZepHr® Impedance/pH Reflux Monitoring System).

24 hour Multichannel Intraluminal pH (MII pH) metry

As described above, impedance is the best technique for detection of reflux while pH metry characterizes acidity. Hence the combination of both techniques is superior to either being used alone (65).

Both pH metry and impedance pH metry catheter systems are attached to a data logger box that is carried by the patient over the 24 hour period for which the catheter is in situ. On completion of the study, the data is downloaded onto a computer and the data is analysed using proprietary software (Sleuth®, Sandhill Scientific, Inc.).

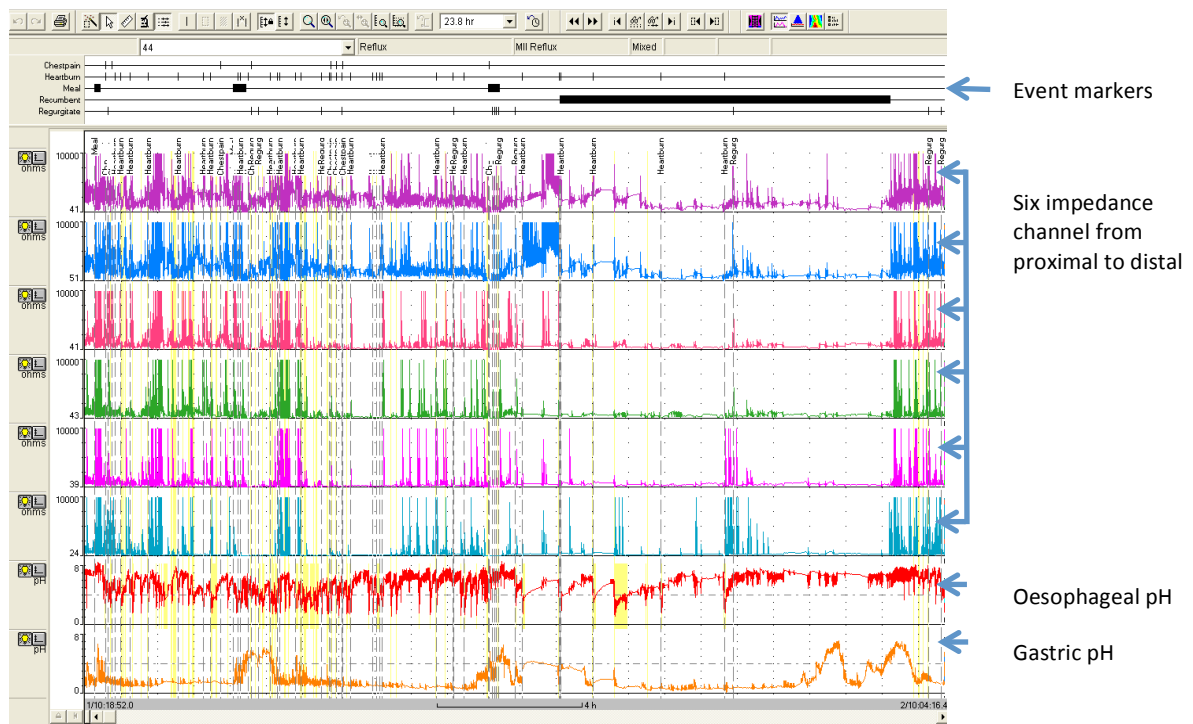


Figure 2.4: Abnormal multichannel impedance pH study with high oesophageal pH levels seen in the oesophageal pH channel and predominantly acidic pH levels seen in the gastric pH channel. This patient had an oesophageal pH of less than 4 for 13.2% of the total study. He had many symptoms and many reflux events.

Reflux monitoring - parameters measured

Percentage of time where distal oesophageal acid exposure to a pH <4 is used to diagnose GORD. A value of distal acid exposure time (AET) of greater than 4.2% over a 24 hour time period is used in the GI Physiology Unit at the Royal London Hospital. The recent Lyon Consensus Group state that a distal AET >6% is conclusive of GORD. Conversely, a distal AET of <4% makes a diagnosis of GORD very unlikely. They recommend that a distal AET of 4-6% on its own is inadequate for making a diagnosis of GORD, and supportive evidence is required in order to make a diagnosis of GORD.

The algorithm used to calculate reflux episodes in impedance pH studies identifies retrograde movement of contents (gas, liquid or mixed). This, in combination with pH of the distal oesophageal pH sensor data is used to identify acid as well as non acid reflux episodes. Impedance pH metry tracings are reviewed manually to ensure artefactual reflux episodes are not included in the final analysis.

The consensus for a pathological number of reflux events is >80 over a 24 hour recording period. 40 or fewer reflux events over a 24 hour period is thought to be physiological, and therefore, 40-80 reflux events alone is inadequate to be classed as pathological per se (125).

On the whole, as mentioned previously, the vast majority of symptoms assessed using the portable data logger, were typical GORD symptoms – heartburn, regurgitation, chest pain and epigastric pain. Atypical symptoms were assessed only occasionally. Patients were instructed on the importance of pressing a symptom button as soon as a particular symptom began. They were instructed to only press the button once for a single episode of a particular symptom. Occasionally a patient would press the button several times in a cluster and in this situation, using a pragmatic approach, the standard practice would be to preserve the first symptom marker of the cluster and to edit out the subsequent markers. If there were several symptom markers and reflux events clustered together, a note would be made on the report of the study as hypervigilance would likely be playing a role the patients symptomatology.

In terms of numbers of symptoms recorded, accurately recorded increased numbers of symptoms increases the statistical significance with regards to reflux symptom association calculations. For the purposes of this study, we used a cut off number of 5 symptom markers per symptom type, although an update of the previous consensus document for the

diagnosis of GORD, the Porto Consensus, recommended that for reliability for reflux symptom association calculations, at least 3 symptom events should be recorded (126).

Reflux symptom association

The most widely used methods for calculating reflux symptom association are Symptom Index (SI) and Symptom Association Probability (SAP), which are both used in our Department, and for the purposes of this study. Other methods of assessing reflux symptom association such as the Symptom Sensitivity Index (SSI) and the Binomial Symptom Index (BSI) have not been used.

The SI was the first method proposed to calculate if a symptom was related to an episode of reflux (127). Here the number of reflux episodes related to a reflux event compared to the total number of symptoms is expressed as a percentage. Relatedness of a symptom to a reflux event is defined as occurring within 2 minutes of a reflux event. Receiver operating characteristic analysis has suggested that the optimal threshold for the SI would be 50% (128). A disadvantage of the SI is the fact that it does not take into account the total number of reflux events.

The SSI is defined as the proportion of reflux episodes that are symptomatic and this is also expressed as a percentage (129). For the SSI 5 or 10% has been arbitrarily used as a cut-off (128). An obvious disadvantage of the SSI is a disproportionately high positivity if the number of recorded symptoms is high.

Because of the complex nature of symptom association, SAP (130) and BSI were developed, which both use more statistically sophisticated methods to calculate reflux symptom association. They both use a $P < 0.05$ as a cut off for positivity, and if positive, the probability of reflux symptom association is unlikely to have occurred by chance.

The SAP is based on a Fisher's exact test performed on a two-by-two contingency table in which the number of two-minute periods with reflux-related symptoms, with reflux-unrelated symptoms, with symptom-free reflux episodes, and with symptom-eliciting reflux episodes is entered. The BSI calculates the probability that symptoms are related to acid reflux by summing the partial probability for each individual symptom that is acid reflux related (128). Of note, the BSI is of more use with longer duration studies. Also, in the context of small number of symptoms, the statistical analysis becomes underpowered, undermining its accuracy.

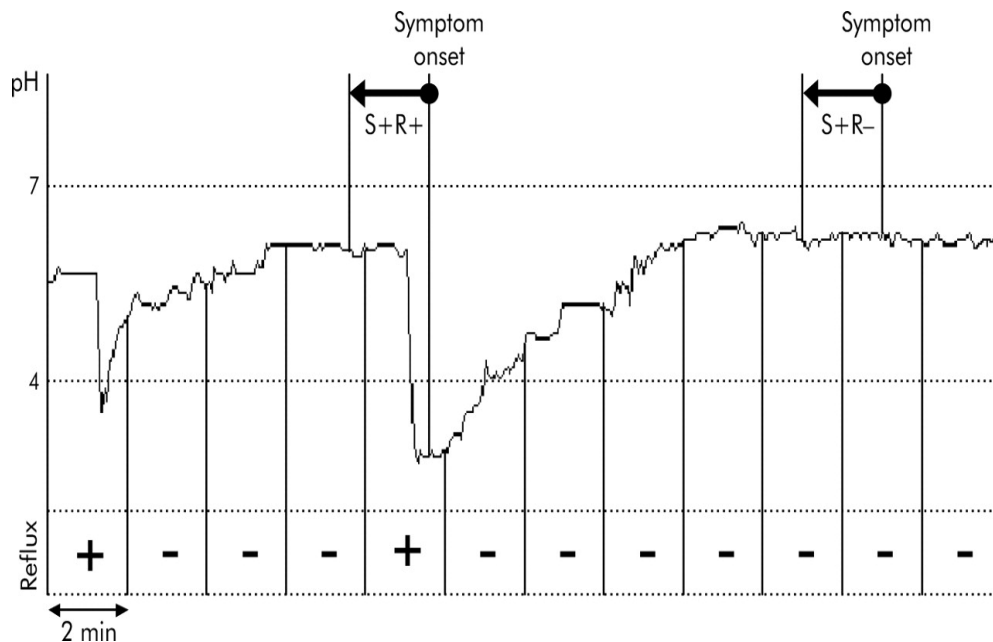


Figure 2.5: The calculation of SAP is represented schematically. The recording is divided into 2- minute segments. Here, two of them contain a reflux episode. The patient has also pressed the symptom button on two occasions denoted by the symptom onset marker. Acid reflux (R) precedes the first symptom (S) (that is, S+R+), but not the second symptom (S+R-).

Previous work on the subject

The first real attempt at characterising the hypersensitive oesophagus category was by Savarino et al in 2008 (131). They prospectively assessed 150 patients with NERD (typical symptoms of GORD and a normal gastroscopy) referred for 24 hour combined MII-pH monitoring. They compared their patient data with that of 48 healthy volunteers. In their population of NERD patients, they identified 87 patients who had physiological levels of distal oesophageal acid exposure. Of these 87 patients, 48 had positive symptom association probability (SAP), which differentiated them from those patients who had a negative SAP and thus a diagnosis of a functional disorder. They further categorised the SAP positivity with acid/non-acid/mixed reflux, demonstrating that patients with a hypersensitive oesophagus do also perceive typical GORD symptoms in response to non acid reflux.

Prior to this, in 2002, Kuran et al demonstrated that on review of 44 patients referred for MII pH monitoring, 7 were classed as having a hypersensitive oesophagus (132).

More recently, Frazzoni et al have demonstrated the use of post reflux swallow-induced peristaltic wave (PSPW) index and the mean nocturnal baseline impedance (MNBI) as a means to characterize RH independently of SAP and SI (133). They assessed MII pH tracings from 125 patients with NERD (defined as gastroscopy negative, PPI-responsive heartburn with an abnormal distal oesophageal acid exposure time), 108 with RH (defined as gastroscopy negative, PPI-responsive heartburn with a normal distal oesophageal acid exposure time), and 70 patients with FH (gastroscopy negative and PPI non responsive with normal distal oesophageal acid exposure time). They showed a lower PSPW index and MNBI in patients with NERD compared to hypersensitive oesophagus. The patients with FH in turn had the highest values. In combination, the area under the curve on receiver operating characteristic analysis was 0.957. The SAP and/or SI was positive in 67 of the 108 RH

patients, but impressively, the PSPW index and/or MNBI was abnormal in 99 of the 108 HE patients (92 %; $P < 0.0001$).

Reflux hypersensitivity at the Royal London Hospital

Study design and objectives

This was a retrospective cohort study conducted in the GI physiology unit of the Royal London Hospital, with a prospective component. Our aim was to assess the incidence of RH in the Gastro-Intestinal Physiology Department at the Royal London Hospital.

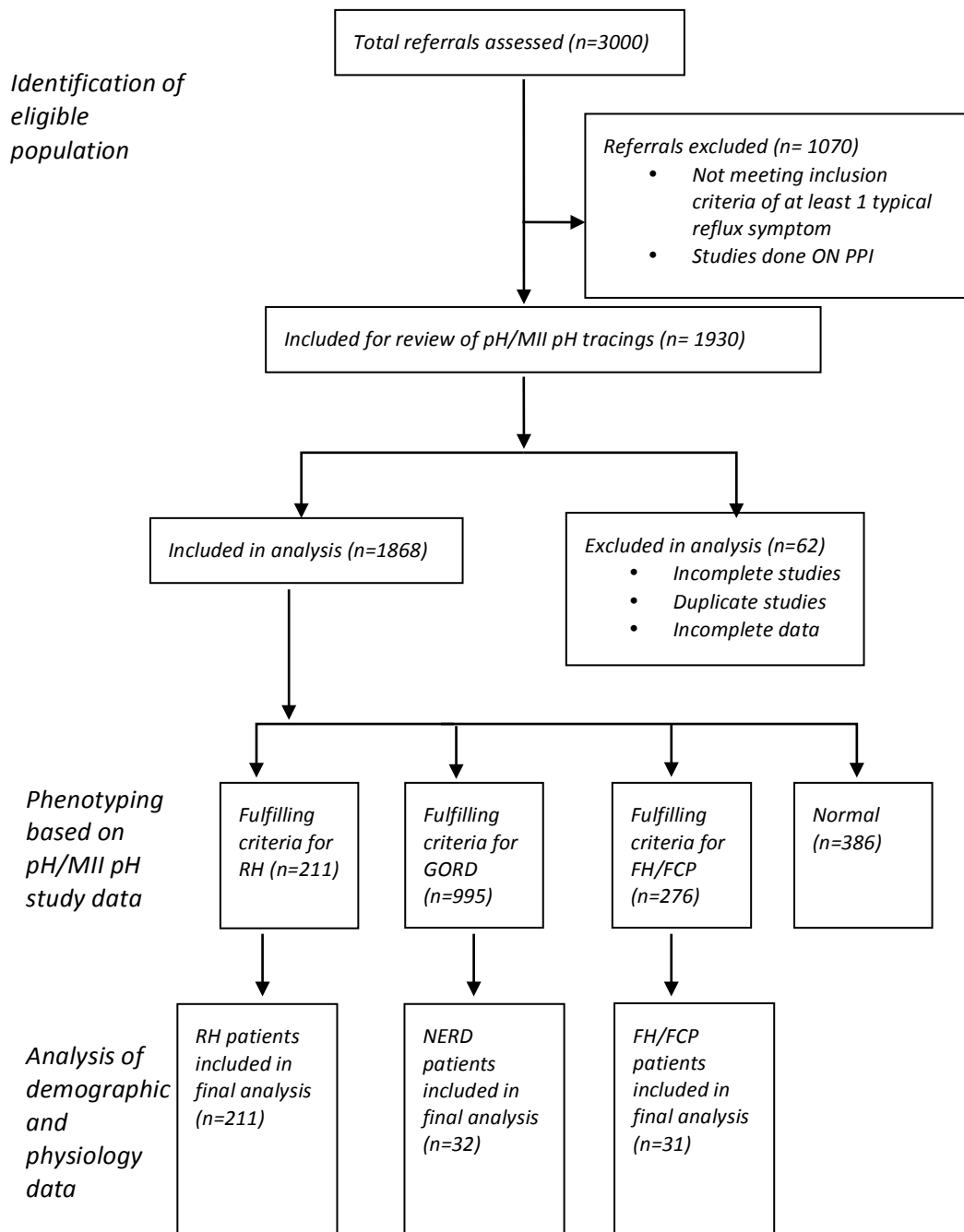


Figure 2.6: Schematic of study design.

Data was collected from an internal database, collated, and ordered by date of tests. Data was checked for accuracy by comparing patient notes and the Central Records System (CRS) of the National Health Service (NHS).

Definitions of pathology specified in terms of pH/MII pH studies

GORD was defined as

- Typical reflux symptoms (heartburn, chest pain, epigastric pain and regurgitation)
- At least 5 recorded episodes of a symptom
- Increased acid exposure time off PPI (>4.2%)

Reflux hypersensitivity was defined as

- Typical reflux symptoms (heartburn, chest pain, epigastric pain and regurgitation)
- At least 5 recorded episodes of a symptom
- Normal acid exposure time (<4.2%)
- Positive SI or SAP

Functional heartburn (FH) and functional chest pain (FCP) were defined as

- Symptoms of heartburn or chest pain respectively
- At least 5 recorded episodes of a symptom
- Normal acid exposure time (<4.2%)
- Negative SI and SAP

A normal study was defined as

- Normal acid exposure time (<4.2%)
- Negative SI and SAP
- Less than 5 episodes of a symptom recorded

Methods

Data ordinarily collected

All patients referred to the GI physiology unit complete a pre procedure questionnaire about demographics, type and frequency of symptoms, as well as past and current medical history and medication history. Before the written informed consent process, the GI physiologist reviews this questionnaire and fills out any missing data before proceeding for safety reasons and to ensure the test is tailored to their symptoms.

Demographic data

The following demographic data are ordinarily collected from patients when they attend the GI physiology Unit as part of service evaluation.

- Age
- Gender
- Ethnicity
- Catchment area (local to Barts and the London NHS Foundation Trust or not)

Medical history data and reflux symptom data

The following medical history and reflux symptom data are ordinarily collected for safety reasons and to ensure the test is tailored to their symptoms.

- Type, frequency and duration of symptoms to be evaluated
- Current medication
- Medical history and medication history
- Presence of oesophageal and extra-oesophageal symptoms
- Weight and height
- Hypermobility score
- Anti-reflux medications such as proton pump inhibitors (PPI) and H2 receptor antagonists (H2RA) used before pH study
- Pain modulator and antidepressant use.

Characteristics of cohort evaluated

All patients included in the study had been referred for assessment of symptoms of GORD from local or regional referral hospitals by a gastroenterologist or a gastrointestinal surgeon. From January 2010 to December 2015, 3000 records of patients referred to the GI Physiology Unit at the Royal London Hospital were identified from the upper GI Physiology Database. Medical records for these patients were assessed based on pH/MII pH data to phenotype them into 4 groups – GORD, RH, FH/FCP and normal using the criteria set out above.

Studies done whilst on acid suppression therapy were not included. Patients with a history of upper gastro-intestinal surgery and severe motility disorders were excluded from the analysis.

Patients with at least one typical symptom of GORD (heartburn, chest pain, epigastric pain and regurgitation) lasting for more than 6 months were included in the analysis. The specific atypical symptoms (in addition to typical reflux symptoms) of throat burning, cough and belching were recorded.

Of note, findings of an oesophago-gastro-duodenoscopy (OGD) were not always available and therefore were recorded only if available. For the patients included in the analysis, a great deal of effort was made to source the OGD result. Those with erosive oesophagitis, Barrett's oesophagus, eosinophilic oesophagitis, oesophageal or upper GI malignancy were excluded from the cohort being studied. The timing of the gastroscopy was not always available and was therefore not recorded. In terms of *Helicobacter pylori* (H pylori), the pattern of where H pylori related gastritis is seen, appears to have a bearing on the extent of acid secretion that occurs. Therefore, this, in combination with other factors such as TLOSRS, appears to contribute to the generation of symptoms of reflux (134). There

is limited evidence assessing the effect of H pylori on oesophageal hypersensitivity and none assessing the effect of H pylori on RH. There was no facility to record H pylori status for any of the patients and therefore I did not evaluate this.

RH cohort

All patient fulfilling criteria for RH were included in the analysis.

NERD and FH/FCP cohort

As shown in the study design section, I selected a cohort of patients with true NERD and with FH/ FCP. The rationale for these two comparison groups was to assess RH in terms of known characteristics of patients with GORD and FH/FCP. Comparison of GORD phenotypes may also inform classification strategies such as ROME IV.

These patients were selected prospectively from the GI physiology database, in a consecutive manner, to avoid selection bias.

32 patients with NERD and 31 patients with FH/FCP were selected out of a group of 995 and 276 respectively. The 2 previous studies presented in the literature (by Savarino et al and Frazzoni et al) both had representative groups of true NERD and FH/FCP. The numbers in my study were comparable to the above studies.

Manometry and pH metry data

None of the studies were longer than 24 hours (ie: pH capsule studies (eg Bravo studies) were not included as they are not routinely performed in our Department. Following completion of high resolution manometry testing and 24 hour pH testing (with or without impedance measurements), the following data were collected. Length of study (excluded if <20 hours as deemed an incomplete study).

- Supine, upright and total acid exposure
- Acid reflux episodes [reflux episodes detected by impedance with a pH <4]

- Non-acid reflux episodes [reflux episodes detected by impedance with a pH>4]
- Total acid reflux episodes
- Proximal reflux episodes
- Reflux diagnoses
- Manometry diagnoses

Statistical analysis

Continuous data that was not normally distributed was analysed using the non parametric Mann Whitney test. Proportions were compared using χ^2 tests. Results were considered statistically significant when $P < 0.05$.

Statistical analysis was carried out using proprietary software (GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com).

Results

Of the patients referred with typical reflux symptoms to our unit, a total of 1869 patients were included in the analysis. 211 patients met the criteria for RH and data was analysed for all 211 of these patients (65% female, mean age 44.4 years, age range 19 to 82, media age 44 years). As control populations, 31 consecutive patients who met the criteria for FH/FCP and 32 patients who met the criteria for NERD were analysed.

- 211 fulfilled criteria for reflux hypersensitive oesophagus,
- 995 fulfilled criteria for GORD (including NERD and ERD),
- 276 fulfilled criteria for functional heartburn and functional chest pain,
- The remaining 386 were deemed to have a normal study, defined as normal acid exposure time (<4.2%), negative SI and SAP or fewer than 5 episodes of a symptom recorded.

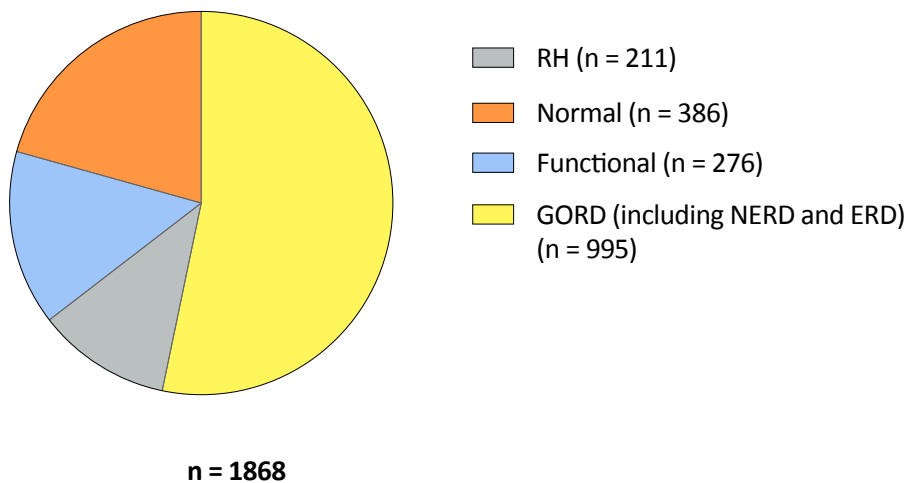


Figure 2.7: Diagnoses of patient referred to the GI physiology unit at the Royal London Hospital with typical GORD symptoms.

Comparison groups

As mentioned in the study design section, I selected a cohort of patients with true NERD and with either FH or FCP. These patients were selected prospectively from the GI physiology database, in a consecutive manner, to avoid selection bias.

The representative cohort of patients from the 995 patients with GORD made up the true NERD group with 32 patients (33% female, mean age 51.15 years, age range 29 to 77, median age 54 years), after review of gastroscopy and pH study results. A representative cohort of patients from the 276 patients identified with FH/FCP made up the FH group with 31 patients (67% female, mean age 51.15 years, age range 18 to 73, median age 47 years).

Gender characteristics

The RH group had a predominance of female patients compared to the NERD group, but a similar percentage of females to the FH/FCP group. As noted above, the majority of patients with RH were female (65%). A similar trend is noted in the FH/FCP group (65%). This is strikingly different from the NERD group, is characterized by male predominance (67%).

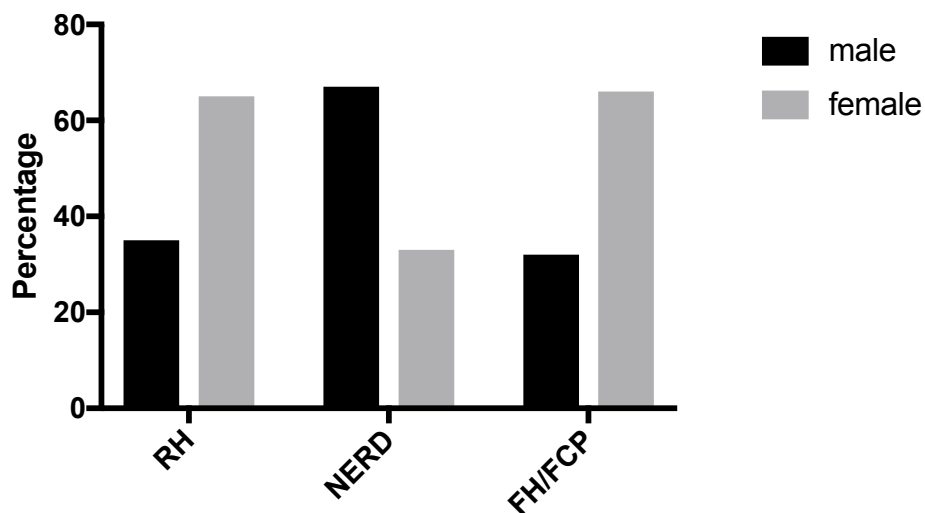


Figure 2.8: Distribution of sex in the three cohorts assessed.

Age characteristics

In terms of age characteristics, the RH group of patients were significantly younger than the NERD group ($p = 0.0089$). There was no significant difference between the age difference between the RH and FH/FCP group ($p = 0.4955$). Patient with RH and FH/FCP in the population I assessed were younger than those with NERD.

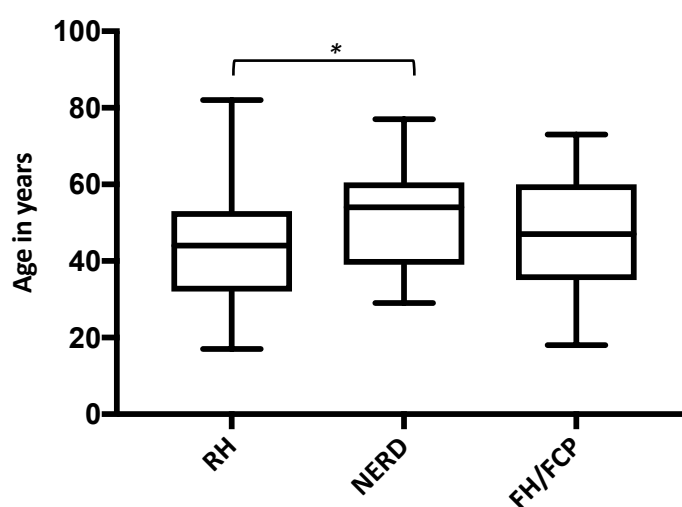


Figure 2.9: Age distribution for the three groups. There was a significant difference in the median age of the RH group compared to the NERD group ($p = 0089$).

Symptom characteristics

Typical reflux symptoms

Distribution of typical symptoms of GORD was evaluated in our RH study population. Data was available for all 211 patients with RH included in the study. All patients volunteered at least 1 typical symptoms of GORD such as heartburn, regurgitation, epigastric pain or chest pain. Heartburn was the most common symptom with 154/211 patients recording it as a significant symptom. This was closely followed by regurgitation, recorded as

a significant symptom by 153/211 patients. 32/211 patients complained of chest pain and 25/211 complained of epigastric or abdominal pain.

In the NERD group, heartburn was the most common symptom with 23/32 patients recording it as a significant symptom. This was closely followed by regurgitation, recorded as a significant symptom by 19/32 patients. 4/32 patients complained of chest pain and 5/32 complained of epigastric or abdominal pain.

In the FH/FCP group, heartburn was the most common symptom with 20/31 patients recording it as a significant symptom. This was closely followed by regurgitation, recorded as a significant symptom by 10/31 patients. 16/31 patients complained of chest pain and 11/31 complained of epigastric or abdominal pain.

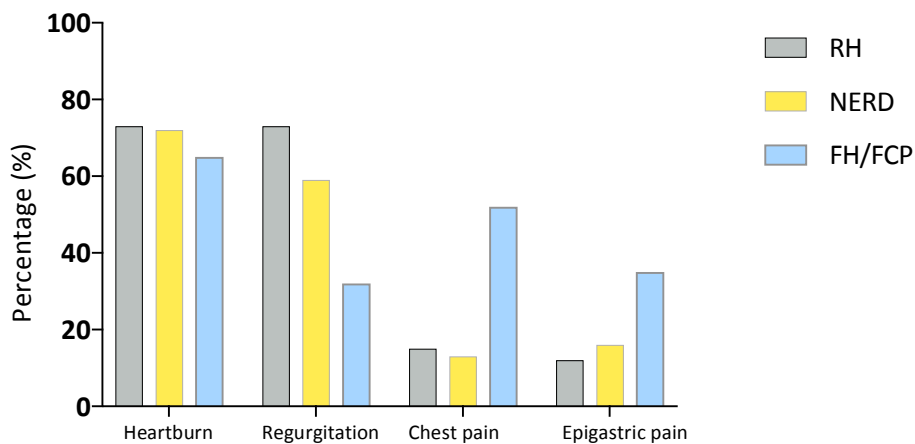


Figure 2.10: Proportion of typical symptoms of GORD experienced by patients in each of the three groups studied, expressed as a percentage.

Reflux characteristics

In the RH group, 35/211 (17%) underwent pH metry alone whilst the rest underwent MII pH metry. In the NERD group, all the patients underwent MII pH metry. In the FH/FCP group, 11/31 (35%) underwent pH metry alone.

Total median distal oesophageal acid exposure (figure 2.11) was 1.3% in the RH group (0.6 – 2.3%), 12.1% (8.35 – 18.35%) in the NERD group and 0.6 (0.2 – 1.4%) in the FH/FCP group. The difference between the RH and NERD group was significant at $p = <0.0001$, whilst the difference between the RH and FH/FCP group was also significant at $p = 0.0048$, confirming that subjects within each of the groups were correctly allocated.

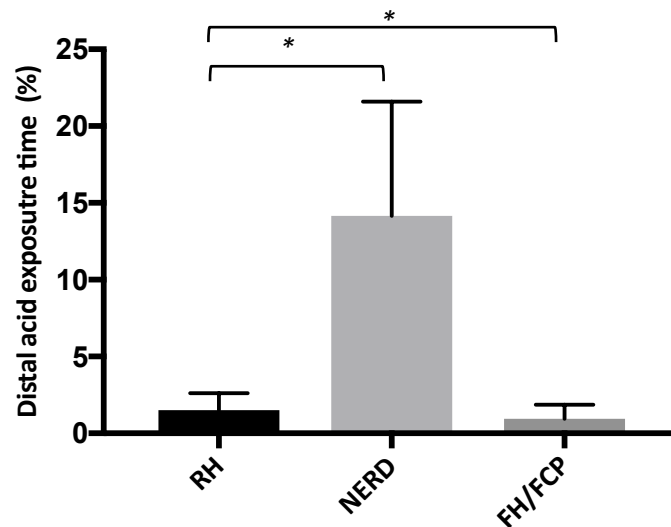


Figure 2.11: Median distal oesophageal acid exposure time was significantly different between the RH and NERD groups and the RH and FH/FCP groups ($p = <0.0001$ and $p = 0.0048$ respectively), confirming that subjects within each of the groups were correctly allocated..

Comparing median supine distal acid exposure, the NERD group had a significantly greater supine distal oesophageal acid exposure at 10% (0.4 – 22.1%) compared to the RH and FH/FCP groups, which had a median supine distal oesophageal acid exposure of 0% (0 – 0.4%) and 0 – 2.4% respectively). Median upright distal oesophageal acid exposure was also significantly higher in the NERD group at 14.1% (8.1 – 18.85%), with the RH group having a median of 2.1% (0.9 – 3.9%) and the FH/FCP group having a median of 1% (0 – 2.4%). This again confirmed that the NERD group experienced pathological distal supine as well as upright acid exposure, as compared with the RH and FH/FCP groups, as expected.

In terms of reflux episodes (figure 2.12), total reflux episodes were recorded for all studies as this is always recorded in both pH metry and MII pH metry studies. Median number of reflux episode during the recording period was 38 (25 – 58) for the RH group and 54 (43 – 75) for the NERD group, which was significantly different ($p = <0.0001$). The FH/FCP group had a median number of total reflux episodes of 28 (12 - 35), which was also significantly different to the RH group ($p = 0.0004$).

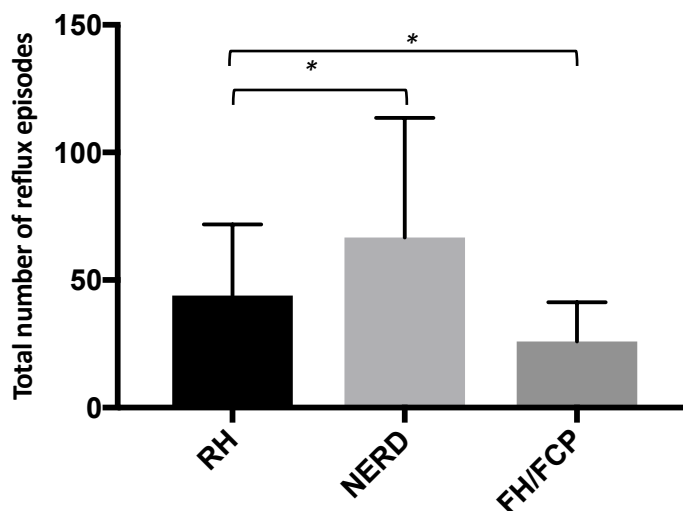


Figure 2.12: Total number of reflux episodes was significantly different between the RH and NERD groups as well as the RH and FH/FCP groups ($p = 0.0001$ and 0.0004 respectively).

When analysing the MII pH data, it is evident that the NERD group have the highest median number of *acid* reflux events (43, 28 - 59), compared to the RH group (18, 8 - 33) and the FH/FCP group (8, 1 - 18). The difference between the numbers of acid reflux events for the RH group compared to the NERD groups is significant ($p = <0.0001$). In fact the difference between the RH and FH/FCP groups is also significant at $p = 0.0019$ (figure 2.13).

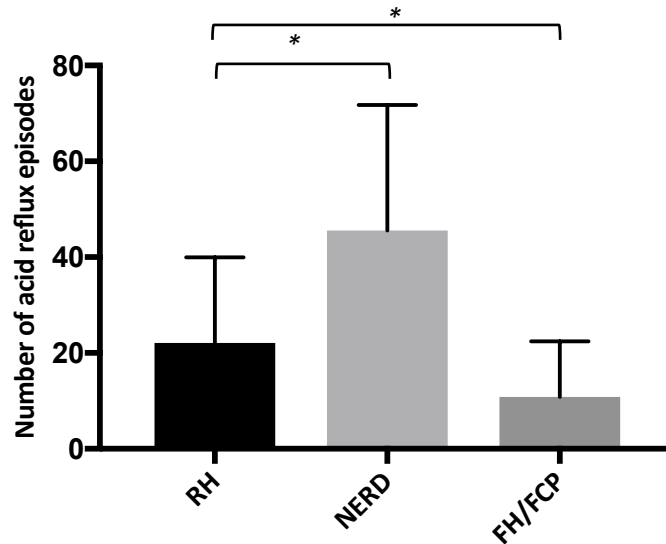


Figure 2.13: Total number of **acid reflux** episodes was significantly different between the RH and NERD groups as well as the RH and FH/FCP groups ($p = 0.0001$ and 0.0004 respectively)

Considering non acid reflux, the RH group had the highest number of non acid reflux events (18, range 0 to 123), compared to the NERD group (12, range 0 to 109) and the FH/FCP group (15, range 1 to 41). The difference between the RH and NERD group was significant at $p = 0.0411$, whilst the difference between the RH and FH/FCP group was not significant ($p = 0.1868$).

	RH	NERD	FH/FCP
Proportion of heartburn episodes	49%	50%	20%
Proportion of regurgitation episodes	43%	34%	11%
Proportion of chest pain episodes	4%	5%	13%
Proportion of epigastric pain episodes	3%	11%	55%

Table 2.1: Proportion of typical symptoms relative to total symptoms in patients with RH ($n = 211$), NERD ($n = 32$) and FH/FCP ($n = 31$).

Dyspeptic symptoms

Patients in the RH group also complained of dyspeptic symptoms, although these were not evaluated as part of the reflux symptom association element of their 24 hour pH or MII pH study. Considering patients with functional disorders are more likely to complain of dyspeptic type symptoms (2), evaluation of the distribution of these symptoms was thought to be of use when attempting to phenotype patients with RH.

- **Bloating**

In our study population, patients with RH complained of bloating and indeed it was a prominent symptom in the RH group, with 66% volunteering it as a symptom. In comparison, NERD patients (63%) had less bloating compared to the RH group whilst patients in the FH/FCP group complained of bloating the most with 84% reporting bloating as a symptom.

- **Belching**

62% of patients in the RH group complained of belching. By comparison, 77% of patients in the FH/FCP group also complained of belching. Interestingly, 79% of patients in the NERD group also complained of belching.

- **Nausea**

Among the RH patient group, 32% complained of nausea. This was similar to the FH/FCP group where 35% had nausea ($p=0.0528$). But only 21% of the NERD group revealed nausea as a symptom, which was significantly lower in comparison to RH ($p=0.0269$).

- **Vomiting**

In the RH group, 29% complained of vomiting as a symptom. This was similar to patients in the FH/FCP groups where 32% complained of vomiting. Vomiting in patients with NERD (24%) was less common than either the RH or FH/FCP groups.

Medications

On review of medications taken by the patients in our cohort, I reviewed acid suppression medications, including proton pump inhibitors (PPIs) and histamine-2 receptor antagonists (H2RAs). In addition to the gastric acid neutralisation effects of these preparations, I also looked at neuromodulator use, in particular, tricyclic antidepressants (TCAs) and selective serotonin receptor inhibitor (SSRI) use.

- **Acid suppressants**

Most patients evaluated were on a PPI, although doses and regimes were varied.

89%, 97% and 90% of patients with RH, NERD and FH/FCP were on a PPI respectively.

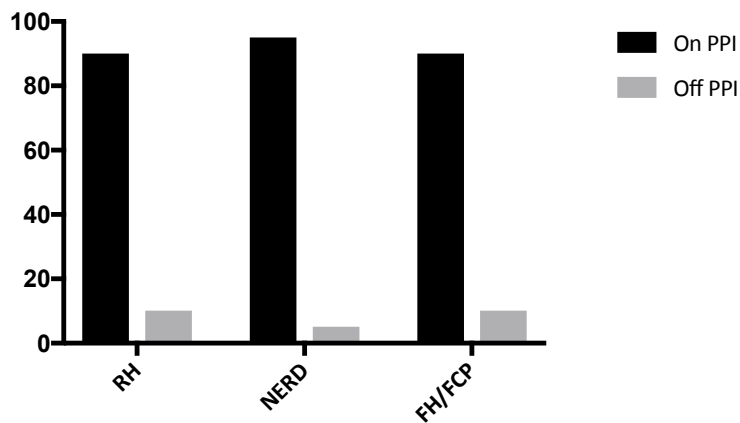


Figure 2.14: Percentage of PPI use in the RH group (n=211), NERD group (n=33) and the FH/FCP group (n=31)

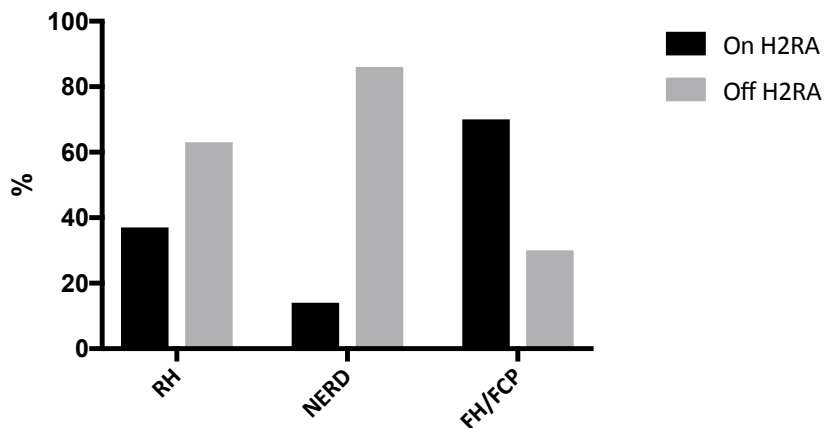


Figure 2.15: Percentage of H2RA use in the RH group (n=211), NERD group (n=33) and the FH/FCP group (n=31)

Use of neuromodulators

Patients with symptoms of GORD refractory to acid suppression therapy unsurprisingly end up being prescribed classes of drugs thought to reduce perception of pain. These commonly include tricyclic antidepressants, SSRIs and pregabalin. 36/211 patients (17%) in the RH group were on one of the above neuromodulation agents (although other reasons for being prescribed these medications cannot be ruled out). In the NERD group 4/33 patients (12%) were taking neuromodulation agents and in the FH/FCP group, this was 7/31 (23%).

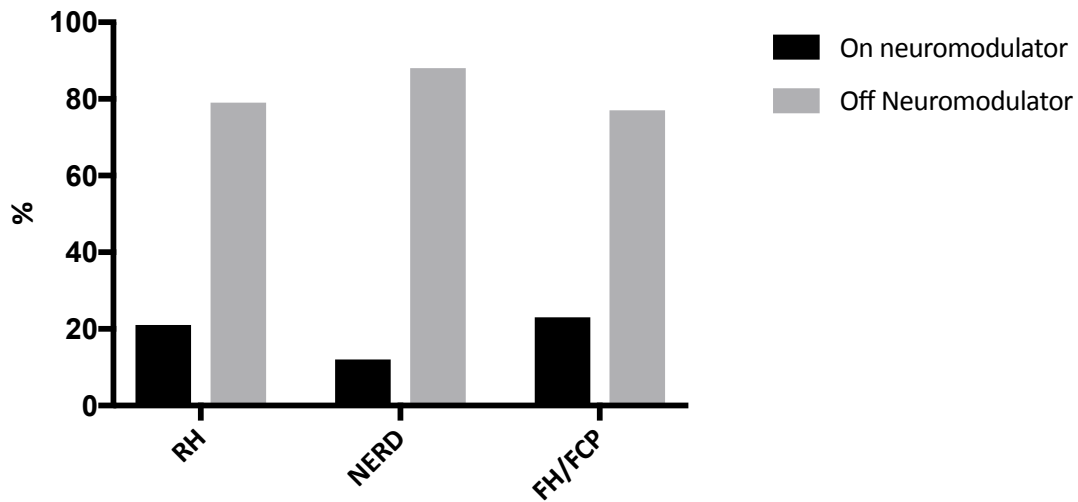


Figure 2.16: Percentage of neuromodulator use in the RH group (n=211), NERD group (n=33) and the FH/FCP group (n=31).

Summary

In summary, I have demonstrated that in our cohort of patients referred for investigation of typical symptoms of GORD, patients with RH account for 11% of our population (211/1868). Patients confirmed as having GORD accounted for 53% and patients with functional heartburn and chest pain accounted for 15%. The male:female ratio in RH was similar to the ratio in FH/FCP as opposed to NERD. They were also significantly younger than the NERD group. The pattern of typical reflux symptoms was however more similar to the NERD group than the FH/FCP group.

Median distal oesophageal acid exposure was significantly higher than the FH/FCP group and significantly lower than the NERD group. Total number of reflux episodes as well as acid reflux episodes was also similarly significantly higher than the FH/FCP group and lower than the NERD group. Distribution of type of typical GORD symptoms showed that RH and NERD patients volunteered a larger proportion of heartburn and regurgitation symptoms, whilst FH/FCP patients predominantly reported symptoms of chest and epigastric pain.

When considering dyspeptic symptoms, patients with FH/FCP complained of more bloating, nausea and vomiting. Belching was seen most in the NERD group.

In terms of medications, most patients in all three groups were on a PPI. Fewer patients were on H2RAs but of the three groups, more patients in the FH/FCP group were on H2RAs compared to the other 2 groups. Neuromodulator use was similar in all three groups, albeit slightly higher in the FH/FCP group.

Discussion

Based on my summary findings, patients with RH have characteristics similar to NERD in some respects and similar to FH/FCP in some respects. Considering the male:female ratio in our cohort, RH had a higher proportion of female patients which is consistent with the literature, in terms of hypersensitive oesophagus, where the consensus based on limited studies have shown a slight female preponderance in RH (135). I have also showed a female preponderance in FH/FCP. Functional chest pain, in the tertiary care setting at least, shows a female preponderance similar to my findings, whereas in the general population, males and females present with functional chest pain equally (136) (137). Functional heartburn is also equally seen in male and female patients. The general perception that more female patients suffer with functional disorders may stem from patterns in other disorders such as irritable bowel syndrome (IBS) (138). Therefore, although the male:female distribution has been evaluated in the literature for GORD and FH/FCP, with some previous work in RH, the male:female distribution in RH has not been previously been evaluated in a group as large as this.

I have shown that heartburn and regurgitation were more significantly prominent symptoms in RH, compared to chest pain and epigastric pain. This has not been evaluated previously and adds to the understanding of the phenotype of the RH patient.

ROME IV clearly defines RH as a separate entity to FH and FCP. In terms of distal oesophageal acid exposure as well as total number of reflux episodes and total number of acid reflux episodes in RH, I have demonstrated that RH is clearly a distinct entity. This may explain the microscopic difference seen in RH when compared to FH/FCP (121) as well as differenced seen in baseline impedance (133). The implication of the results for non acid reflux are not easy to explain. It may be that the increased proportion of acid reflux compared to non acid reflux in patients with NERD allows for the relatively higher

proportion of non acid versus physiological acid reflux in RH to appear as significant, when in fact this is not so. Comparing the RH group with a group of healthy volunteers in terms of non acid reflux and physiological acid reflux may help clarify the significance of non acid reflux in this case. The non-significance of non-acid reflux episodes between the RH and FH/FCP group may does not really inform us further either, and a comparison with a groups of healthy volunteers may be useful here as well. Either way, non acid reflux may be less significant than has been suggested in previous studies (139).

When considering dyspeptic symptoms, again the RH group in our cohort were similar to the NERD group with 66% vs 63%. The FH/FCP group however complained of significantly more bloating with 84% volunteering bloating as a bothersome symptom. Nausea and vomiting, although not common were seen in marginally more patients in the RH and FH/FCP group compared to the NERD group. The contribution of visceral hypersensitivity in the pathophysiology of all three conditions (2) is likely to explain these not insignificant levels of dyspepsia in all thee groups studied.

Belching however was clearly more common in patients with NERD, which is possibly due to the contribution of TLOSRS in the pathophysiology of reflux (66). The levels of belching in RH and FH/FCP were not insignificant though at 62% and 77% respectively. Belching was not specifically investigated during impedance metry for the vast majority of patients, although a few who complained of significant belching affecting quality of life were reviewed in terms of belching during their MII pH study. The predominant rationale for this was to look for potential supragastric belching, especially since our unit was trialling behavioural therapy as a treatment for problematic supragastric belching (140).

Despite being on a PPI, all patients still complained of at least 1 typical symptoms of GORD to qualify for inclusion into this study, hence referral to a tertiary centre for further investigation of their symptoms. This fact reinforces the need for improvement in the

diagnosis and treatment for those patients who are either partially or fully refractory to acid suppression therapy.

Increasing evidence for the use of SSRIs and gabapentin in FH and FCP (4) has led to increased prescribing of these medications when clinicians suspect a functional component to the symptoms experienced by a patient. Despite this however, it is interesting to note that despite 23% of the FH group already being on either amitriptyline, an SSRI or pregabalin, they presumably did not have sufficient relief of their symptoms, culminating in a tertiary referral for further investigation of their symptoms.

The body of this work represents the largest group of patient studied with RH, previously termed hypersensitive oesophagus, as classified by ROME III criteria. Although ROME IV reclassified RH as a functional oesophageal disorder as opposed to a subtype of NERD, the only real change in terms of diagnostic criteria was the fact that specific reflux symptom association parameters were removed, although evidence of “triggering of symptoms by reflux events despite normal acid exposure on pH or pH-impedance monitoring” forms part of the current diagnostic criteria. This is currently not quantified in the ROME IV consensus document, which is unusual in a parameter driven environment that surrounds the study of GORD. The rationale given is the uncertainty of the “true clinical value” of reflux symptom association. In effect however, confirming that a symptom is “triggered” by a reflux event is a component of reflux symptom association. Therefore our cohort of patients still falls within the diagnostic criteria for RH.

As would be expected, there are some concerns including those raised by Frazzoni et al, that RH is better characterised as it was previously, as a subtype of NERD (141) and they argue that the classification of RH as a functional disorder is not justified at present. Their argument proposes that newer impedance parameters such as the PSPW index and MNBI, in addition to reflux symptom association would better define patients with RH. Of note, the

same authors have demonstrated that both these parameters have been shown increase diagnostic yield in NERD (142). This highlights the need for better phenotyping of patients diagnosed with RH.

In summary, it is clear that my findings contribute to the understanding of RH and inform the following chapters in this thesis. This work also affords improved phenotyping of patients, which is especially useful when assessing novel therapies for the treatment of GORD and oesophageal pain.

Chapter 3

*Treatment of oesophageal hypersensitivity in patients with
NERD: effect of a prostaglandin EP1 receptor antagonist*

Introduction

The pathophysiology of symptom generation in NERD is thought to involve central as well as peripheral sensitisation. Pathological and also physiological acid exposure to the distal oesophagus is thought to sensitise local nerves (possibly via dilated intercellular spaces) as well as dorsal horn nuclei, leading to increased sensitivity of the distal and also the proximal oesophagus to acid (94).

In general, assessment of oesophageal sensitivity in NERD patients has yielded evidence for reduced perception thresholds for painful stimuli. Miwa et al evaluated stimulus response functions to acid in patients with NERD, compared with ERD and FH, using an acid perfusion model. They demonstrated that NERD patients had lower perception thresholds for pain, especially compared with normal controls, but also compared to those with erosive oesophagitis and Barrett's oesophagus (143).

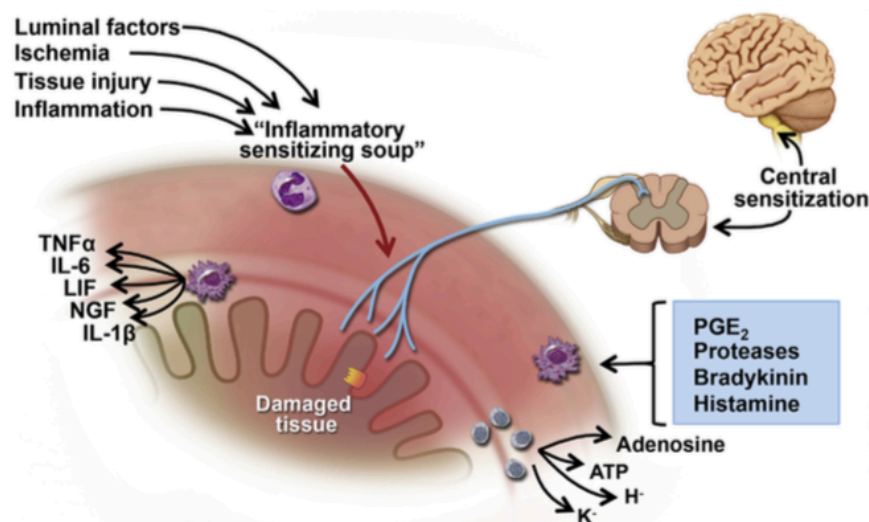


Figure 3.1: Mechanisms underlying sensitization. Luminal factors and mediators released in response to ischemia, injury, and inflammation act on the sensory endings to drive sensitization. These peripheral mechanisms are reinforced by central mechanisms in the spinal cord and CNS. ATP, adenosine triphosphate; LIF, leukaemia inhibitory factor; NGF, nerve growth factor; PGE, prostaglandin E; TNF, tumour necrosis factor (94).

Several mediators involved in this process of oesophageal pain hypersensitivity have been identified recently, including prostaglandin E₂, TRPV1, PAR-2 and ASICs as discussed in chapter 1 of this thesis (24-27). Identification of such target mediators and a means of manipulating them in order to treat oesophageal pain comprises one strategy to address the gap in therapies for patients with refractory GORD.

In this chapter, I investigated the effect of a compound that is a potent and selective prostaglandin EP1 receptor antagonist as a therapy for oesophageal pain in patients with NERD.

Prostaglandin E₂

The prostanoid prostaglandin E₂ (PGE₂) is an important mediator of both central and peripheral sensitisation and the prostaglandin E receptor 1 (EP1) appears to have a major, but not exclusive, role in mediating the contribution of PGE₂ to both peripheral and central sensitisation (144-147). PGE₂ exerts its cellular effects through four different G protein-coupled receptors encoded by separate genes, termed EP1, EP2, EP3 and EP4 (148). Among the four subtypes, EP1 receptors appear to have a major role in processing of pain (146, 149).

ONO-8539

ONO-8539 is a potent and selective prostaglandin EP1 receptor antagonist developed by ONO Pharmaceutical Co., Ltd. Preclinical data generated in a distal oesophageal acidification model in the monkey demonstrated that ONO-8539 is effective in increasing pain tolerance threshold following electrical stimulation of the proximal oesophagus. Their data suggested that ONO-8539 might therefore be an effective modulator of oesophageal pain hypersensitivity. Previous clinical studies have shown it to be safe and well tolerated in humans (150). The most frequently reported GI-related adverse event was

diarrhoea and the majority of adverse events seen were mild and resolved without intervention (151).

Study design

This was a randomised, two centre (London, UK and Leuven, Belgium), double-blind, placebo-controlled, two-period, crossover study design. The main objective was to evaluate ONO-8539 on a model of oesophageal pain hypersensitivity in patients with NERD. Subjects with a confirmed diagnosis of NERD on a stable dose of PPI were enrolled into the study. ONO-8539 or placebo was dosed as an add-on therapy to a stable dose of PPI. ONO Pharmaceutical designed the protocol, in collaboration with the Principal investigators of both sites and their respective research groups. I was involved in the latter part of the refining process.

Each subject was randomised to ONO-8539 300 mg bid or placebo bid in the first treatment period and the alternate treatment in the second treatment period. Each treatment period lasted 28 days (± 1 day). There was a washout of ≥ 13 days between each treatment period. Subjects were randomised to treatment sequence in a 1:1 ratio. Subjects were enrolled for a total of approximately 19 weeks from the screening visit (visit 1) to the follow-up visit (visit 10) and attended the clinic for a maximum of 10 visits. Subjects were outpatients and therefore no overnight stays were required.

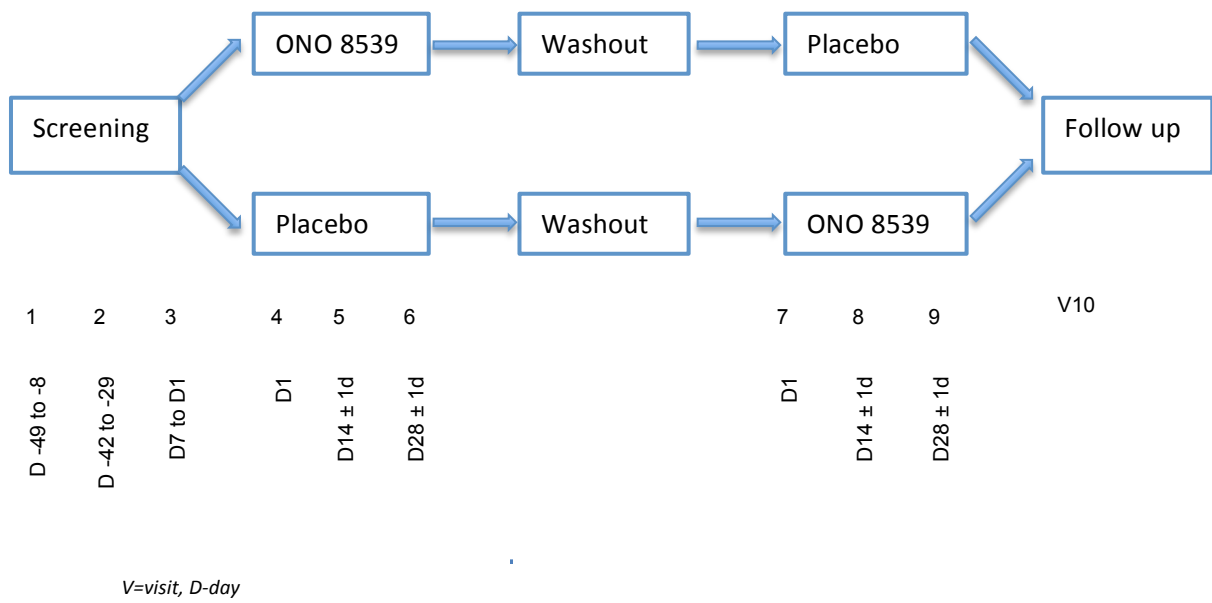


Figure 3. 2: Schematic of study design.

Study medication

The study medication consisted of either 300mg of ONO-8539 or matching placebo, that was taken orally with water twice-daily. As ONO-8539 is a weak inhibitor of the cytochrome P450 (CYP) isoenzyme CYP3A4, a list of contraindicated medications was formulated to ensure co administration was avoided, thus reducing the risk of inadvertently increased drug levels.

Medications affecting PGE₂ levels such as non-steroidal anti-inflammatory drugs, COX-2 inhibitors and Prostaglandin drugs were also prohibited during the study.

Inclusion and exclusion criteria

As expected with a clinical trial involving an investigational medicinal product, the inclusion and exclusion criteria were extensive and rigorous.

Inclusion criteria

1. Able to give written informed consent.
2. Male or female subjects aged 18 to 70 years inclusive.
3. Confirmed GORD according to the Montreal definition (1)
4. Confirmed normal oesophageal mucosa on endoscopy.
5. On a stable dose of a PPI for at least 4 weeks prior to Visit 4.
6. Moderate intensity heartburn on at least 2 days a week for at least 8 weeks prior to Visit 3, as reported by the patient on direct questioning.
7. Scoring of intensity of their symptoms at least 'very mild' (≥ 5.3 cm) following distal oesophageal acid perfusion using a previously validated verbal descriptor scale.
8. If male, an agreement to use a highly effective method of contraception from Visit 4 until 3 months after their follow up study visit.
9. If female, and of child-bearing potential, an agreement to use a highly effective method of contraception for a period of at least 28 days before Visit 4 until at least 1 month after the follow up visit.

10. The subject was able to meet the study restrictions.
11. The subject was capable of independently completing the study diaries and questionnaires.

Exclusion criteria

1. Any present or past history of any significant disease or disorder that would increase the risk for the subject if they were enrolled in the study, or would affect study procedures or outcomes.
2. Presence of oesophageal motility disorders, as identified by HRM at screening.
3. Inability to tolerate oesophageal acid perfusion, oesophageal electrical stimulation or oesophageal intubation.
4. A history of GI, renal or hepatic disease, prior endoscopic anti-reflux procedure, major GI surgery or any other condition that may have interfered with drug absorption, distribution, metabolism or excretion.
5. Normal oesophageal acid exposure (total time pH<4 less than or equal to 4.2%) during 24 h MII-pH monitoring, and both a negative SI and SAP.
6. Any acute gastro-intestinal symptoms within 14 days of Visit 4 with the exception of GORD symptoms and constipation.
7. An identified endoscopic or manometric abnormality.
8. Any clinically medical issue within 4 weeks of Visit 4 which in the opinion of the Investigator would place the subject at undue risk, could influence the results or ability of the subject to participate in the study.
9. A history of alcoholism or drug abuse.
10. A clinically significant laboratory abnormality.
11. Cardiovascular conditions within 6 months prior to Visit 1. Abnormal blood pressure unless well controlled. Prolonged QT interval.
12. A positive drugs of abuse, hepatitis B, hepatitis C or human immunodeficiency virus (type I or II) test at Visit 1.
13. A history of hypersensitivity to any of the drug constituents as listed in the investigators brochure (IB).
14. Participation in a clinical trial involving an investigational medicinal product (IMP) within 3 months or five half-lives of the IMP (whichever is longer) of Visit 1.
15. Unable to use a PPI or on any contraindicated medication that could influence the efficacy of the PPI or the ability to continue their use of a PPI.

16. Taking any prohibited concomitant medication within the defined period prior to screening Visit 1.
17. Changing the dose of a permitted medication required to be at a stable dose, within the defined period prior to screening Visit 1.
18. Taking any medication, which in the opinion of the Investigator would place the subject at undue risk, could influence the results or ability of the subject to participate in the study.
19. Donating blood and/or received blood or blood products within the previous 3 months prior to screening Visit 1
20. Pregnancy, lactating or planning to become pregnant during the course of the study.
21. Inadequate vision or manual dexterity to complete the subject diary.
22. Difficulty swallowing tablets.
23. Unable to cooperate fully with study staff, difficulty following some study requirements, or otherwise not qualified for the study.
24. Previously received ONO-8539.
25. Vulnerable, imprisoned or institutionalized by regulatory or court order.

Study Objectives

Primary Objective

The primary objective of the study was to evaluate the effect of ONO-8539 on oesophageal pain hypersensitivity to oesophageal acid perfusion.

Secondary Objectives

1. To evaluate the effect of ONO-8539 on subject-reported symptoms of GORD (severity and frequency)
2. To assess the safety and tolerability of ONO-8539

Exploratory Objective(s)

1. To investigate the effect of ONO-8539 on oesophageal pain hypersensitivity to electrical stimulation
2. To evaluate the effect of ONO-8539 on quality of life
3. To investigate the pharmacokinetics of ONO-8539

Methods

Baseline data collection

The following demographic data and medical history were taken at screening.

Baseline assessments were also conducted.

- Date of birth, gender, race
- A full medical history, including GORD symptoms, medication history, smoking history, alcohol consumption history and systemic enquiry.
- Endoscopy
- HRM and MII pH monitoring
- Physical examination (including weight and height)
- Vital signs and 12-lead ECG
- Clinical laboratory tests (including viral screen)
- As part of screening, baseline oesophageal pain threshold was recorded using electrical stimulation of the oesophagus.
- Baseline response to oesophageal acid exposure was recorded using a Modified Bernstein test
- Baseline personality trait, anxiety levels, symptom scores, quality of life scores as well as fear of pain scores are recorded.

Study procedures

Endoscopy

Gastroscopy was performed at screening to review the presence of mucosal abnormalities in the upper GI tract.

Oesophageal Manometry and 24 hour MII pH monitoring

Oesophageal manometry was undertaken at screening. Motility disorders were reviewed with regards to inclusion and exclusion criteria. The upper border of the LOS was recorded. A 24 hour MII-pH study was also undertaken to confirm NERD and exclude FH and FCP.

Oesophageal pain threshold measurement

Oesophageal electrical stimulation was performed using a bespoke catheter with a distal oesophageal electrode. The electrode was placed 5cm above the LOS and oesophageal pain threshold was determined by increasing the electrical stimulation intensity in steps of 2 mA, until the subject first reported pain. The mean of three separate measurements was recorded as the pain threshold. The duration of the electrical stimulus pulse was 200µs and the frequency of presentation 0.5 Hz.

Modified Bernstein Test

Several studies investigating various aspects of GORD, use the Bernstein test, or modifications of it, to investigate the effect an agent on the perception of acid (or non acid) reflux (152-154). The modification used in this study was the use of the perception of “slightest discomfort” instead of “typical symptoms” as an endpoint for the test.

Prior to starting the modified Bernstein test (MBT), a standard instruction script was read out in order to standardise instructions. During the MBT, saline of 0.9% was initially infused into the oesophagus through an infusion port 10cm above the LOS at a rate of 10ml/minute. After 2 minutes, the infusion was changed to one on 0.1 M Hydrochloric acid (HCL) at a rate of 10ml/minute, for a period of 10 minutes. The patient was unaware of the switch and was asked to alert the investigator when they first felt “the slightest discomfort”.

Quantification of stimulus-response to acid

This was quantified by three parameters:

- Lag time to symptom perception (defined as the time, in seconds, to initial discomfort perception following the MBT).
- Sensory intensity rating (an assessment of the intensity of symptoms at the end of the MBT, made using a previously validated verbal descriptor scale.).
- APSS (acid perfusion sensitivity score)

The APSS was calculated from lag time (T) expressed in seconds (sec) and sensory intensity rating (I) expressed in centimeters (cm). The figure obtained is divided by 100 for convenience: $APSS = (I) \times (T)/100$ (cm x sec/100) (152-155).

The verbal descriptor scale consisted of a 20-cm vertical bar flanked by descriptors of increasing intensity (no sensation, very weak, faint, weak, very mild, mild, moderate, barely strong, slightly intense, strong, intense, very intense, extremely intense). Placement of words along the scale was determined from their relative log intensity rating in a normative study. The validity of these scales for assessing the perceived intensity of visceral sensation has been previously confirmed (156, 157).

The APSS was chosen as a suitable endpoint in view of previous validation studies where it was utilised by Fass et al to assess chemosensitivity to acid in healthy volunteers and patients with GORD (152). They also used it to assess chemosensitivity to acid in older and younger patients with GORD (155) more recently they used it to evaluate the response of patients with GORD to auditory stress (158). In this last study, the APSS was able to clearly differentiate GORD patients from healthy volunteers at baseline, and after the active intervention versus the control.

Questionnaires

Subjects were asked to complete several questionnaires at defined time points during the study to assess symptoms, anxiety and depression.

Reflux Symptom Questionnaire 7 Day Recall

This is a well-validated questionnaire used in patients with GORD who experience only a partial response to PPI therapy. It is brief and easy to complete and is intended for use in routine clinical care. It consists of 13 items incorporating oesophageal and extra oesophageal symptoms of GORD, and requires the patient to document frequency and intensity of symptoms over the previous 7 days. It is frequently used in clinical trials assessing new therapies for the treatment of GORD and it was therefore a suitable questionnaire to study the effect of treatment on symptoms of GORD (159).

State-Trait Anxiety Inventory

This is a widely used self-report questionnaire to assess anxiety, which has good reliability and has been validated extensively (160). As one may expect, the state questionnaire asks how the subject feels at the present moment, whereas the trait questionnaire enquires about longer term feelings of anxiety. The subject was required to select how closely they identified with 20 different emotions at that point in time on a scale of 1 (“not at all”) to 4 (“very much so”). The Trait Anxiety Questionnaire examines longer-term traits toward anxiety than the State Anxiety Questionnaire. The subject was required to select how frequently they associated with 20 different traits on a scale of 1 (“almost never”) to 4 (“almost always”).

Patient Health Questionnaire–15

This questionnaire is self-administered and comprises 15 somatic symptoms derived from the full Patient Health Questionnaire. Each symptom is scored 0 (“not bothered at all”),

1 (“bothered a little”), or 2 (“bothered a lot”). The PHQ-15 examines how much subjects have been affected by the 15 different somatic symptoms over the previous 4 weeks. The results of this questionnaire indicate if the subject is experiencing somatisation disorder and at what level (161).

Hospital Anxiety and Depression Scale

The HADS is a short self-administered questionnaire that takes approximately 5 minutes to complete. It is designed to screen medical patients for anxiety and depression and is well validated (162). There are seven items on the questionnaire relating to anxiety and seven relating to depression. The subject indicated how closely they related to each, and a score of 0–3 is generated for each answer. An overall figure (out of 21) is generated for both anxiety and depression (163), with higher scores corresponding to higher levels of anxiety and depression.

Fear of Pain Questionnaire (FPQ)

The FPQ is a self-administered assessment which outlines different scenarios in which a subject could potentially experience pain and asks how much they would fear the pain in each scenario on a scale of 1 (“not at all”) to 5 (“extreme”) (164).

Quality of Life Assessment - Inhibition (QOLRAD-RI)

This is a well-validated questionnaire used in patients with dyspepsia and heartburn predominant reflux (165), (166), (167). It measures the effect of upper gastro-intestinal symptoms over the previous week on various aspects of quality of life including emotional well-being, sleep, vitality, eating and drinking and physical and social functioning (166).

Statistical Methods

Determination of sample size

The sample size for this study was selected based on feasibility, as this was an exploratory study. As there was no defined and accepted pharmacodynamic endpoint in this population, considering the mechanism of action of ONO-8539, there was a paucity of information to facilitate establishing formal powering. Thus, a sample size of 30 subjects was selected on the basis of comparability to other exploratory studies in this population in the literature. Assuming a withdrawal rate of between 10–20%, this was still considered sufficient to provide data to assess the pharmacodynamics of ONO-8539 as an exploratory study.

Statistical analysis

Continuous variables are presented as mean and standard deviation, or medians and inter-quartile ranges, dependent on data distribution. Categorical variables were summarised by the mean and standard deviation.

ONO Pharmaceuticals carried out statistical analysis on pre-defined sets. This data was reanalysed by me as well. The full set analysis (FAS) comprised all randomised subjects who received ≥ 1 dose of study drug and who had ≥ 1 valid post-randomisation pharmacodynamic evaluation. The FAS was the secondary analysis population for this study and it was used for analysis of the primary endpoint. The per protocol set (PPS) included all subjects in the FAS who did not deviate from any major entry criteria, did not deviate from the protocol between randomisation and study completion and met criteria for compliance to the study drug. The PPS was defined on a period-by-period basis. In addition an overall PPS was defined that included subjects in both the PPS for treatment period 1 and the PPS

for treatment period 2. The PPS was the primary analysis population for pharmacodynamic and quality of life endpoints.

The primary endpoint of change from baseline in the APSS was due to be analysed using analysis of covariance (ANCOVA) to compare the results in the ONO-8539 group with those in the placebo group. This analysis was due to be performed on the PPS. The primary analysis was also due to be repeated for the lag time and sensory intensity rating. It should be noted that because the target sample size was not achieved, it was felt that statistical analysis of the primary endpoint would be underpowered and therefore differences were described instead.

The secondary endpoint was based on the PPS. Absolute value and change from baseline were summarised descriptively by treatment and time-point (baseline and end of treatment period).

Analyses performed used proprietary software (GraphPad Prism version 7.00 for MAC, GraphPad Software, La Jolla California USA, www.graphpad.com). $P < 0.05$ was adopted as the level of statistical significance.

Results

Demographic characteristics

Although it was planned to randomise a total of 30 subjects, due to recruitment difficulties only 14 subjects were randomised to treatment. All these subjects were recruited from the Queen Mary University of London site. Due to the length and invasive nature of the study, local recruitment was difficult and so an amendment was sought to advertise City wide in the printed media. Just under 2000 subjects were screened by a research nurse and myself in order to randomise the above 14 subjects.

All subjects received at least 1 dose of study drug. 2 subjects discontinued the study prematurely (sciatica requiring NSAID therapy and mild ECG abnormality referred to cardiology).

Overall, the median age of subjects was 53.0 (33-62) years. Most subjects were male (8 subjects [57.1%]) and white (10 subjects [71.4%]). The median BMI was 28.030 (18.70-47.00) kg/m². Demographic characteristics were similar between the 2 treatment sequences. All patients included in the analysis fulfilled the inclusion and exclusion criteria. Of note, they were confirmed to have a negative OGD as well as a positive 24 hour MII pH study (12 subjects confirmed as pathological GORD and 2 as RH).

Compliance data

Median compliance was 100% for ONO-8539 and placebo. With respect to PPI, median compliance was 96.55% in both the ONO-8539 and placebo groups. Six subjects used lansoprazole (at doses of 30 mg daily [4 subjects], 15 mg twice daily [1 subject], or 15 mg three times per day [1 subject]), 5 subjects used omeprazole (at doses of 20 mg once daily [3 subjects] or 20 mg twice daily [2 subjects]) and 1 subject used esomeprazole (at a dose of 40 mg twice daily).

Oesophageal hypersensitivity assessments

Acid perfusion sensitivity score (APSS)

The primary endpoint in this study was the change from baseline in the APSS, following oesophageal acid perfusion after 28 days of treatment.

At baseline, the mean APSS was higher in the ONO-8539 treatment group at 75.424 (± 35.154) compared with the placebo group at 61.391 (± 28.471).

After 28 days of treatment, the mean change from baseline in APSS showed a directional change in favour of ONO-8539; however, the magnitude of the change was very small in both treatment groups, -15.471 (± 48.144) in the ONO-8539 treatment group and -12.622 (± 30.790) in the placebo group. An analysis of the change from baseline (adjusted for baseline) showed a smaller change in the ONO-8539 treatment group and a greater change in the placebo group (LS (least squares) means: -11.8 and -16.3, respectively); the differences between treatments were not statistically significant ($p=0.757$). The mean APSS at baseline was higher in treatment period 1 than in treatment period 2.

The APSS data obtained in this study were comparable with those obtained in NERD patients in previous studies (152).

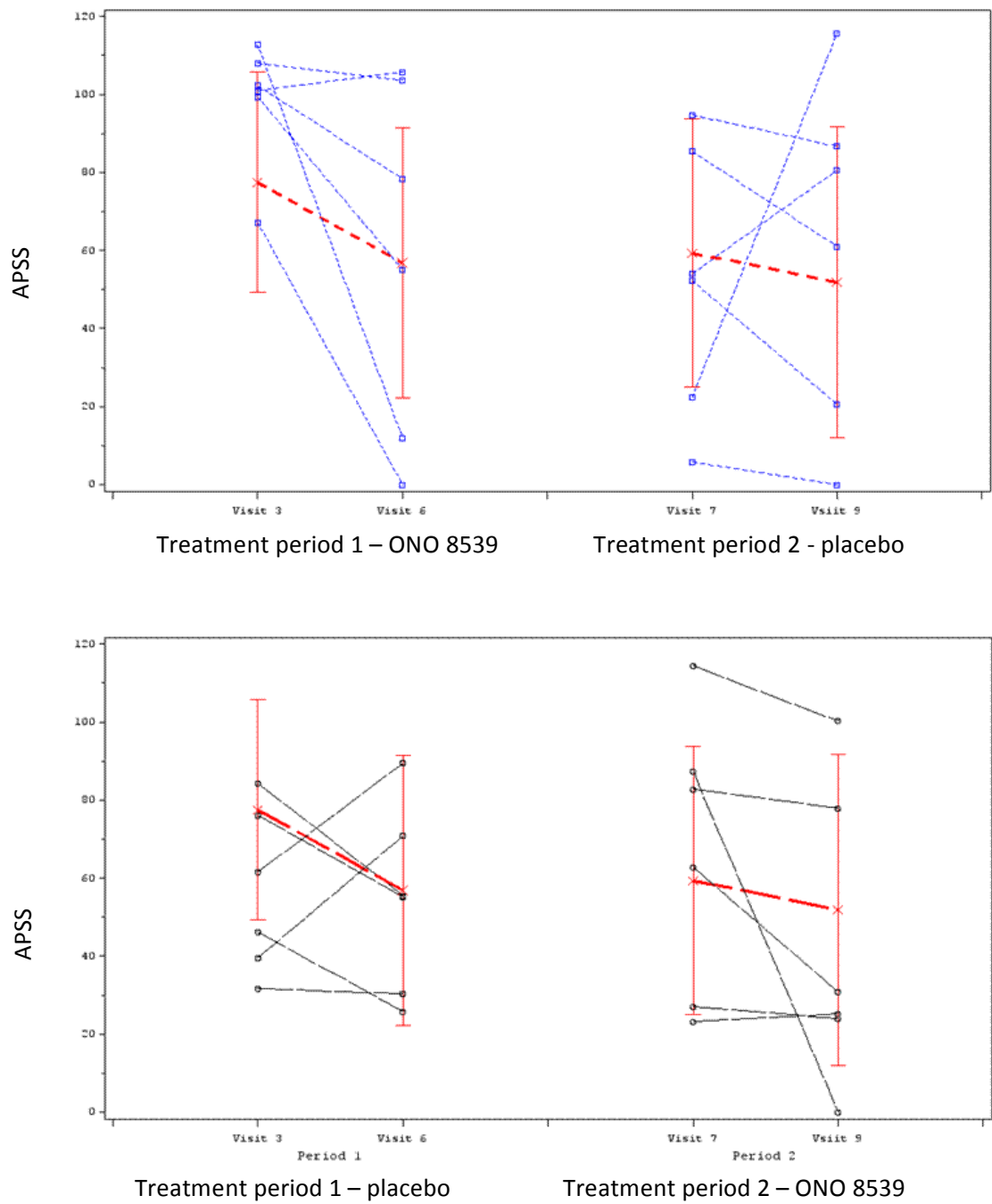


Figure 3. 3: This plot illustrates the lack of a consistent response in APSS within each treatment group. The large within and inter subject variability is demonstrated as well. The data in red indicate the mean (SD) at each visit.

Sensory intensity rating

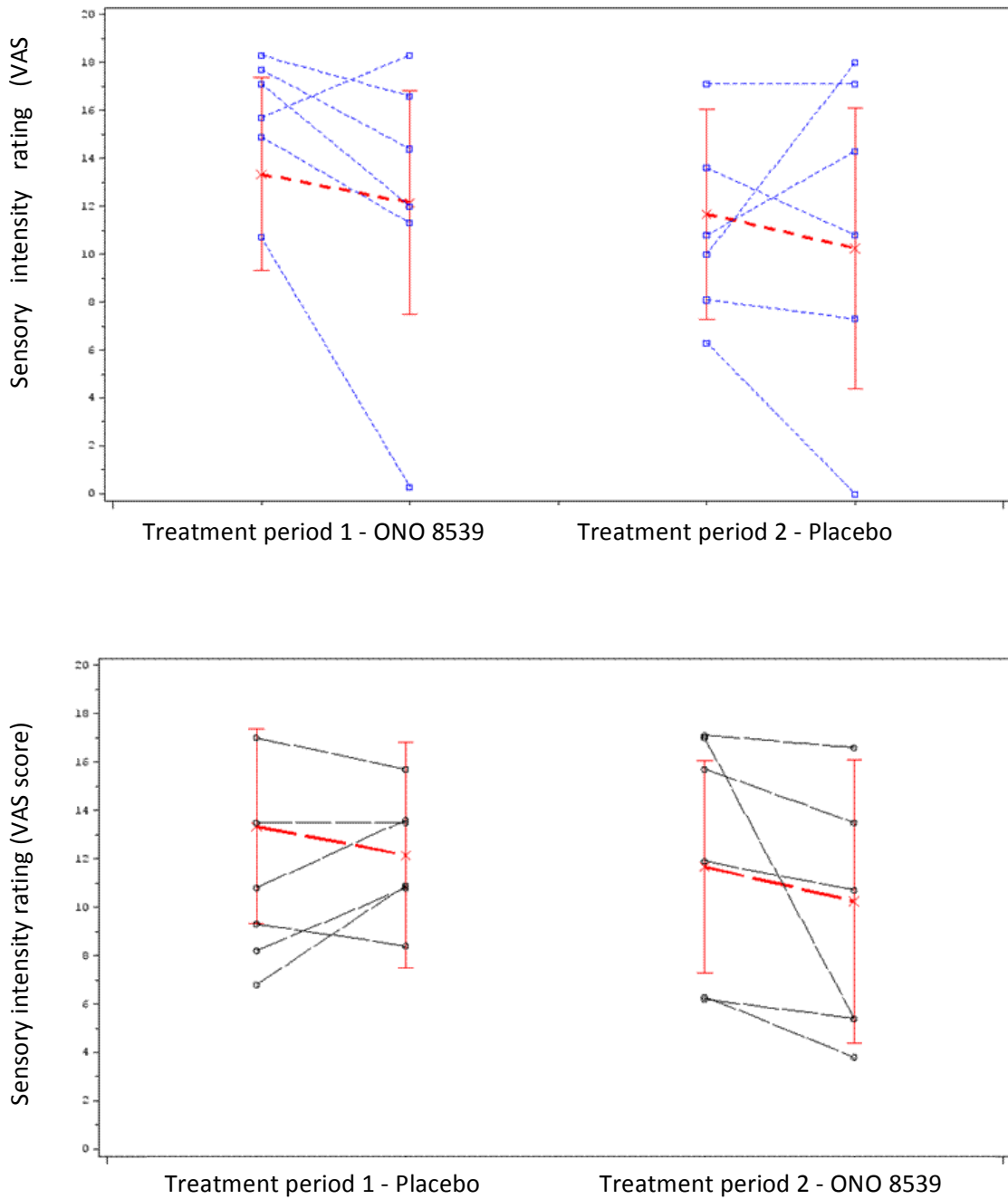


Figure 3.4: This plot illustrates the lack of a consistent response within each treatment group in sensory intensity rating. A large within and inter subject variability is also demonstrated.

The data in red indicate the mean (SD) at each visit.

At baseline, the mean sensory intensity rating was higher in the ONO-8539 treatment group at 13.36 (± 4.06) cm compared with the placebo group at 11.65 (± 4.34) cm. After 28 days of treatment, the mean change from baseline in sensory intensity rating showed a directional change in favour of ONO-8539; however, the magnitude of the change was small in both treatment groups, -1.66 (± 4.85) cm in the ONO-8539 treatment group and -0.96 (± 3.94) cm in the placebo group. An analysis of the change from baseline (adjusted for baseline) had similar results (LS means: -1.6 cm in the ONO-8539 treatment group and -1.0 cm in the placebo group). The differences between treatments were not statistically significant ($p=0.742$).

In addition, the mean sensory intensity rating at baseline was higher in treatment period 1 than in treatment period 2, suggesting an effect of learning on the baseline values between treatment period 1 and 2. Thus, there appeared to be an order effect.

Lag time

At baseline, the mean lag time was shorter in the ONO-8539 treatment group at 66.2 (± 183.51) sec compared with the placebo group at 90.5 (± 74.53) sec. After 28 days of treatment, the mean change from baseline in lag time showed a directional change in favour of ONO-8539 at 118.3 (± 286.12) sec in the ONO-8539 treatment group and 73.1 (± 214.12) sec in the placebo group. An analysis of the change from baseline (adjusted for baseline) had very similar results (LS means: 110.0 sec in the ONO-8539 treatment group and 81.5 sec in the placebo group). The differences between treatments were not statistically significant ($p=0.779$).

In addition, similar to mean baseline sensory intensity rating, the mean lag time at baseline was lower in treatment period 1 than in treatment period 2, also suggesting an order effect.

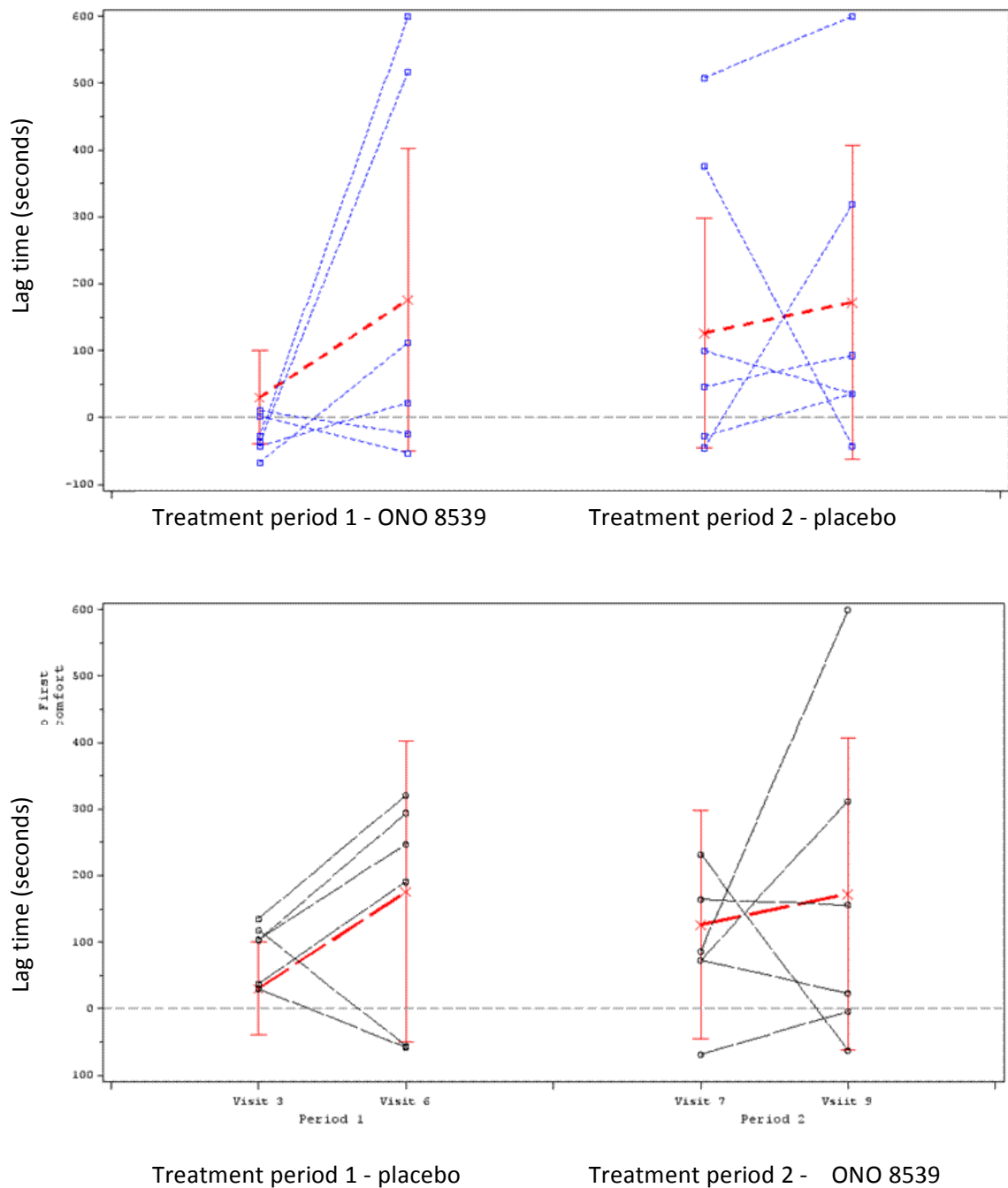


Figure 3.5: This plot illustrates the lack of a consistent response within each treatment group in sensory intensity rating. Similar to the previous two stimulus response measures, a large within and inter subject variability is demonstrated. The data in red indicate the mean (SD) at each visit; a negative time indicates that the discomfort occurred during the saline perfusion.

Reflux symptom questionnaire analysis

Changes from baseline in each of the four RESQ-7 domains (i.e., burping, cough, heartburn and regurgitation; each assessed for frequency and intensity) were generally greater following treatment with ONO-8539 compared with placebo, although none of the changes were statistically different compared with placebo. Of note, mean change from baseline in burping frequency score was -1.25 (± 1.631) following treatment with ONO-8539 compared to -0.33 (± 1.451) following treatment with placebo ($p=0.270$). Burping intensity score was -1.33 (± 1.155) following treatment with ONO-8539 versus -0.58 (± 1.782) following treatment with placebo ($p=0.159$).

No clear pattern was observed in relation to the changes in the RESQ-7 categories. None of the observed differences in change from baseline in RESQ-7 categories were statistically significantly different between treatments.

Pain threshold analysis

None of the observed differences in change from baseline in pain threshold were statistically significantly different between treatments ($p=0.409$).

Pharmacokinetic analysis

Mean pre-dose ONO-8539 plasma concentration was 100.608 (± 104.6350) ng/mL for the mid study period (week 2) and 86.083 (± 58.3468) ng/mL at the end of the study period (week 4). These plasma concentrations observed were consistent with results from previous studies with ONO-8539 (151).

Quality of life, anxiety, depression and health questionnaire analysis

None of observed differences in change from baseline in any of the QOLRAD-RI domains (i.e., emotional distress, food/drink problems, physical/social functioning, sleep disturbance and vitality) were statistically significantly different between treatments ($p=0.545$, $p=0.942$, $p=0.949$, $p=0.411$ and $p=0.857$, respectively).

At baseline, the STAI-T was lower in the ONO-8539 treatment group (36.0) compared with the placebo group (39.5); the STAI-S was higher in the ONO-8539 treatment group (36.7) compared with the placebo group (33.8). No clinically relevant changes in mean STAI-S scores were observed on Day 28 compared to baseline following treatment with ONO-8539 or placebo. The difference in change from baseline in total STAI-S score was not statistically significantly different between treatments.

None of the observed differences in change from baseline in PHQ-15 questionnaire score were statistically different between treatments ($p=0.371$). None of the observed differences in change from baseline in HADS anxiety score were statistically different between treatments ($p=0.128$). Overall, the mean total fear of pain (FPQ) score was lower in the ONO-8539/placebo treatment sequence (57.7 (± 11.84)) compared with the placebo/ONO-8539 (62.7 (± 11.45)). Individual subject responses to the FPQ showed a high degree of variation.

Response rates to measured endpoints

The number of responders for APSS, sensory intensity rating and lag time were generally higher following treatment with ONO-8539 compared with placebo.

Although the mean changes from baseline tended to demonstrate a directional change in favour of ONO-8539 compared with placebo, the effect of ONO-8539 on APSS, sensory intensity rating and lag time was not statistically significantly different from placebo

when adjusted for baseline. A similar pattern was also observed in secondary and exploratory analyses.

The number of responders for APSS, sensory intensity rating and lag time were generally higher following treatment with ONO-8539 compared with placebo. However, differences between treatment groups in the numbers of responders were small, and only one of the differences was statistically significant. When considering the number of responders with a 50% reduction in lag time, there were significantly more responders during treatment with ONO-8539 than during placebo.

Changes in all 4 RESQ-7 domains (burping, cough, heartburn and regurgitation; each assessed for frequency and intensity) were generally greater following treatment with ONO-8539 (i.e., indicating a greater improvement versus baseline) compared with placebo although none of the differences were statistically or clinically significant.

None of the changes in QOLRAD-RI, STAI-S, PHQ-15 and HADS (with the exception of HADS depression) questionnaire scores were statistically significantly different following treatment with ONO-8539 compared with placebo.

	<i>ONO-8539</i> N=12 n (%)	<i>Placebo</i> N=12 n (%)	<i>p-value</i>
<i>APSS % reduction from baseline</i>			
30%	5 (41.7)	4 (33.3)	0.656
40%	5 (41.7)	3 (25.0)	0.395
50%	4 (33.3)	2 (16.7)	0.237
<i>Sensory intensity rating % reduction from baseline</i>			
30%	2 (16.7)	2 (16.7)	0.775
40%	2 (16.7)	1 (8.3)	0.634
50%	2 (16.7)	1 (8.3)	0.634
<i>Time from start of acid perfusion to first perception of the slightest discomfort % reduction from baseline</i>			
30%	4 (33.3)	4 (33.3)	0.970
40%	4 (33.3)	3 (25.0)	0.498
50%	4 (33.3)	1 (8.3)	0.042
<i>Sensory intensity rating scale AND lag time, %reduction from baseline</i>			
30%	2 (16.7)	1 (8.3)	0.799
40%	2 (16.7)	1 (8.3)	0.799
50%	2 (16.7)	1 (8.3)	0.799

Table 3.1: Summary of Responders – PPS.

Adverse Events

The overall incidence of total adverse events (TEAs) was numerically higher after treatment with ONO-8539 (11 subjects [84.6%]) compared with placebo (8 subjects [61.5%]). The majority of TEAEs were mild in intensity (headache, nausea and mild abdominal discomfort). ONO-8539 300 mg bid administered for 28 days was shown to be safe and well tolerated.

Summary

This randomised, double-blind, two centre, placebo-controlled, two-period, crossover study in subjects with a diagnosis of NERD was designed to investigate the effect of ONO-8539 on oesophageal pain hypersensitivity to acid perfusion using a MBT.

Mean decreases in APSS and sensory intensity rating, and mean increase in lag time to symptom perception demonstrated a directional change in favour of ONO-8539 compared with placebo. The magnitude of the change seen was very small in both treatment groups, and none of the differences were statistically significantly different compared with placebo when adjusted for baseline. At baseline, APSS and sensory intensity rating were higher and lag time was lower in the ONO-8539 treatment group compared with placebo.

A large within and inter subject variability was observed in the change from baseline in APSS, sensory intensity rating and lag time. When change from baseline in APSS, sensory rating and lag time was analysed per treatment period, the effect of ONO-8539 was greater during treatment period 1 than in treatment period 2. A similar pattern was observed across secondary and exploratory analyses, where a directional change in favour of ONO-8539 was observed, but only one of the observed changes from baseline was statistically significant. For the number of responders with a 50% reduction in lag time, where there were significantly more responders during treatment with ONO-8539 than during placebo.

Exposure to ONO-8539 300 mg bid in this study was consistent with previously obtained human data at this dose and a directional trend was evident between higher plasma concentrations of ONO-8539 and greater improvement in APSS, sensory intensity rating and lag time (151).

Discussion

It was initially planned to randomise a total of 30 subjects, however, due to recruitment difficulties, only 14 subjects were randomised and 12 subjects completed the study (and were included in the PPS). Therefore, although this was an exploratory study, the study was likely underpowered to detect any statistical differences between treatments. The reduced sample size led to a number of statistical analyses not being performed.

Recruitment difficulties were likely due to several reasons. Firstly, the invasiveness of the study (4 MBTs over the entire study period) was a significant factor. Aside from attempting to avoid the order effect mentioned above, reducing the number of invasive components of a study such as this is likely to improve recruitment as such a study may be perceived as more appealing. This would also have an added advantage of shortening the length of the study, which may also help improve recruitment. Recruitment was affected by the extremely tight inclusion and exclusion criteria as well as study restrictions specified in the protocol for this study. In order to preserve the integrity of the scientific method, a careful balance must be achieved between applying a pragmatic approach and trying to achieve the most carefully phenotyped idealistic study population. Perhaps in this study, reducing the number of endpoints would have allowed for fewer restrictions and facilitated a more achievable set of inclusion and exclusion criteria.

Although the use of the MBT has been reported in the literature, its use as a primary endpoint in a therapeutic intervention study is limited. Therefore, the sensitivity of this endpoint to detect differences between active and placebo treatment in oesophageal pain hypersensitivity is not fully understood. In addition to this, some patients were not able to complete all 12 minutes of the MBT, which is likely to have had a bearing on the VAS sensory intensity score, and therefore the APSS.

The APSS itself has limitations in terms of being a function of two different subjective stimulus responses. Therefore, if either lag time or sensory intensity rating are influenced by factors that have not been controlled for, the reliability of the APSS is diminished. This is therefore a potential argument to support the use of one stimulus response such as lag time as a stimulus response measure instead of APSS.

Lag time has its limitations as well, as perception of “slightest discomfort” might be seen as more subjective compared to, for example pain tolerance threshold to electrical stimulation. That said, I was not able to show a difference in pain tolerance threshold before and after each treatment period for either treatment.

The placebo response in this study was high which is not unexpected in a group of subjects with a high unmet medical need. High placebo responses have been previously demonstrated in pain studies (168) and several factors are recognised to increase the magnitude of the placebo response (e.g., number of tablets taken, subjects’ expectations that they would see an effect, clinicians’ warmth, prestige, and positive attitude). The placebo effect, paired with the reduced sample size, likely compromised the ability to demonstrate whether or not ONO-8539 demonstrated a clear therapeutic benefit over placebo.

I have demonstrated what appeared to be an order effect in this study with a difference in baseline observed between treatment period 1 and 2. A learning effect linked to subjects becoming more aware of what to expect during the MBT could not be excluded as the subject’s became more accustomed upon repeating the MBT. This has a significant bearing on how the MBT is used in studies evaluating oesophageal pain. Previous studies using the MBT have at most used 2 MBTs one day apart (152) or in quick succession (169). These studies did not assess for an order effect as a single baseline was used. Thus, the use

of several MBTs over the course of a single study had not been tested previously, which was a significant limitation of this study.

As per inclusion criterion 7, subjects had to rate the intensity of their symptoms as at least very mild (5.3 cm); however, there was no restriction relating to the lag time, which may explain the greater difference in baseline lag time compared with the difference in baseline intensity. This may therefore have accounted in part to the variability in the response seen in the study for lag time, which in turn likely contributed to the lack of ability to observe statistical differences between ONO-8539 and placebo.

Chapter 4

A randomized single blinded crossover study to investigate the effect of physiological modulation of the ANS using transcutaneous vagal nerve stimulation on oesophageal pain hypersensitivity in healthy volunteers

Introduction

As described in this thesis thus far, GORD is recognised as a significant cause of morbidity, healthcare seeking and reduction in quality of life (38, 39). In particular, patients who have NERD, who are refractory to current available therapy (to varying extents) represent a sizable proportion of patients with GORD (3).

In chapter 1, I discussed pharmacological, electrical stimulation and mechanical therapies for GORD, as well as therapies for RH and functional oesophageal disorders. I also discussed the therapeutic gap for patients who are refractory to current therapies, hence the importance of evaluating newer therapies to address the symptoms experienced by these patients.

Autonomic modulation in visceral pain perception

Patients with both true NERD (with no evidence of oesophageal injury at gastroscopy, and pathological distal oesophageal acid exposure on 24 hour pH studies) and oesophageal RH often display heightened sensitivity to intra-oesophageal stimuli, which is referred to as oesophageal pain hypersensitivity (170).

The proposed pathophysiology of this visceral pain hypersensitivity includes –

- peripheral sensitization of primary afferent nerves at the site of injury and
- central sensitization where repeated stimulation of peripheral nociceptors, leads to sensitization of spinal dorsal horn neurons, with an increase in the receptive field of these neurons, leading to hypersensitivity in areas remote from the area of injury.

The experience of oesophageal pain however, is highly individual with a multitude of factors proposed to account for this variability, in particular, dysfunction of the ANS (171).

The ANS is thought to play a critical role in the modulation of pain through its interactions

with the nociceptive system at the level of the periphery, spinal cord, brainstem and forebrain (172, 173).

Oesophageal pain hyperalgesia due to distal oesophageal acid infusion is correlated with an increase in sympathetic tone (as measured by a rise in cardiac sympathetic index and skin conductance response), as well as a reduction in parasympathetic tone (measured by cardiac vagal tone and cardiac sensitivity to the Baroreflex) (174). Ness et al demonstrated that low intensity vagal nerve stimulation reduced pain threshold to cutaneous thermal stimulation (175). Botha et al demonstrated that deep slow breathing prevented oesophageal hyperalgesia due to an increase in parasympathetic tone, with a corresponding decrease in sympathetic tone, an effect that was reversed by the vagolytic drug atropine (176). Iovino et al demonstrated that by increasing sympathetic tone per se (using lower body negative pressure, which produces venous pooling in the lower body, and a subsequent circulatory response as a result of sympathetic activation), they were able to show significantly increased visceral sensitivity (demonstrated as increased perception of duodenal distention) compared to somatic stimulation (transcutaneous electrical stimulation of the dorsum of the non dominant hand) (177).

Thus, increasing PNS tone has a broadly anti-nociceptive effect whilst increasing SNS tone appears to have pro-nociceptive effects. Thus, raising PNS tone is more likely to cause a general anti nociceptive effect than an organ specific effect. It is also likely then that a balance of sympathetic and parasympathetic tone is required for "normal" pain perception, with wider reaching impacts on visceral pain and inflammation in particular (178).

The oesophageal pain hypersensitivity model

As mentioned in Chapter 1 of this thesis, our group has developed and validated a human model of oesophageal pain hypersensitivity in healthy volunteers (103), where distal oesophageal acidification reduced pain threshold to electrical stimulation in the non acid exposed proximal oesophagus. Sharma et al used this model to demonstrate an increase in sympathetic tone as well as a decrease in parasympathetic tone, in response to distal oesophageal acid infusion (174). Botha et al then used this model to demonstrate that increasing parasympathetic tone using slow deep breathing had an anti-nociceptive effect, with a reduction in oesophageal pain hyperalgesia caused by distal oesophageal acid perfusion (176).

Vagal nerve stimulation

Stimulation of neurons using electrical impulses or magnets has been used in various circumstances, from deep brain stimulation to sacral nerve stimulators. The stimulus alters function or properties of the synapse, and although the exact mechanism of such neuro-modulation is not entirely clear, there is evidence to suggest that parasympathetic activation may be a contributing factor (175).

In addition to this, there is evidence to show that neurotransmitter concentrations at the synapse are altered, with excitatory or inhibitory effects. In particular, there is evidence to show that levels of noradrenaline and gamma-Aminobutyric acid (GABA) are increased by vagal nerve stimulation (179). The significance of this lies in the role of noradrenaline and GABA in neuromodulation of cognitive processes such as memory and perception.

Development of new circuits or even changing the output of existing circuits has been described (180). Many neurons have ionotropic and metabotropic receptors to the

same neurotransmitters; therefore modification of either of these types of receptors is also likely to have an effect on neurotransmitter release, and, therefore neuronal activity (181, 182).

Neurostimulation has also been shown to activate neuronal reflex circuitries, a phenomenon known as the cholinergic anti-inflammatory pathway, which is mediated by the vagus nerve and the $\alpha 7$ subunit of the nicotinic acetylcholine receptor expressed on cytokine producing cells (183). Activation of this pathway by electrical stimulation of the vagus nerve or administration of $\alpha 7$ selective drugs, is effective in ameliorating inflammation by reducing TNF α and IL6 levels, improving survival in experimental models of sepsis, haemorrhagic shock, pancreatitis, postoperative ileus and endothelial cell activation (183).

Vagal nerve stimulation is well established as a therapy for conditions such as epilepsy (14) and depression (15). The evidence for its use in other conditions such as sepsis and inflammation as described in the previous paragraph, is emerging (18). In the context of pain, vagal nerve stimulation has been used to treat fibromyalgia (19) and migraine (20) although the mechanisms of the anti-nociceptive effect here is not well elucidated. As mentioned above, vagal nerve stimulation may be a viable means of increasing parasympathetic tone, harnessing this anti-nociceptive effect for the treatment of pain.

Vagal nerve stimulating devices

Vagal nerve stimulation devices are made up of a power supply, a programmable electrical pulse generator, and electrodes. Stimulation parameters depend on the particular device, and validated protocols have been developed for example in epilepsy. Most vagal nerve stimulators approved for use in epilepsy at present are implanted stimulators, similar to cardiac pacemakers, with the electrode usually wrapped around the left cervical vagus nerve (119) (figure 4.1). Use of the right vagus has been shown to reduce heart rate due to right vagal innervation of the sino atrial (SA) node in rats, so, the left vagus is stimulated preferentially, although not exclusively (184).



Figure 4.1: Implanted vagal nerve stimulator

More recently however, an external transcutaneous VNS (t-VNS) system, consisting of an earplug-like electrode to interface with the concha of the outer ear, and a handheld battery-powered electrical stimulator, has become commercially available (NEMOS device www.cerbomed.com) (figure 4.4). This device stimulates the cymba conchae of the ear for the treatment of epilepsy, and it has been shown to be safe and well tolerated with a high

degree is user friendliness (185). It has also been used in a preliminary study investigating the effect of tVNS on pain perception (186).

Auricular branch of the vagus nerve

The cymba conchae of the ear is used due to the innervation of this area by the auricular branch of the vagus nerve. The cymba conchae of the ear is almost exclusively supplied by the auricular branch of the vagus nerve (187) (figure 4.2 and figure 4.3). This therefore is an easily accessible area to place a tVNS electrode in order to stimulate the vagus nerve. Functional magnetic resonance imaging (fMRI) studies have confirmed similar patterns of cerebral activation with both tVNS of the cymba conchae and implanted vagal nerve stimulators (188).

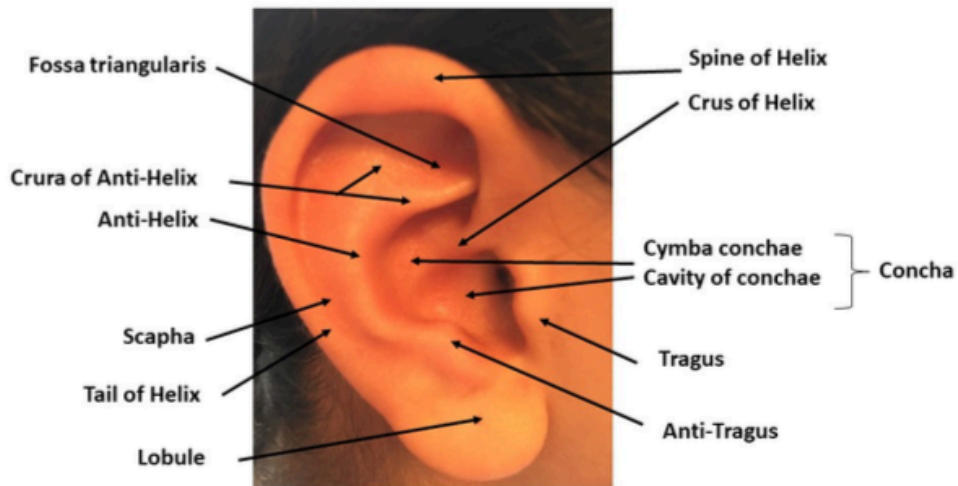


Figure 4.2: Surface anatomy of the ear

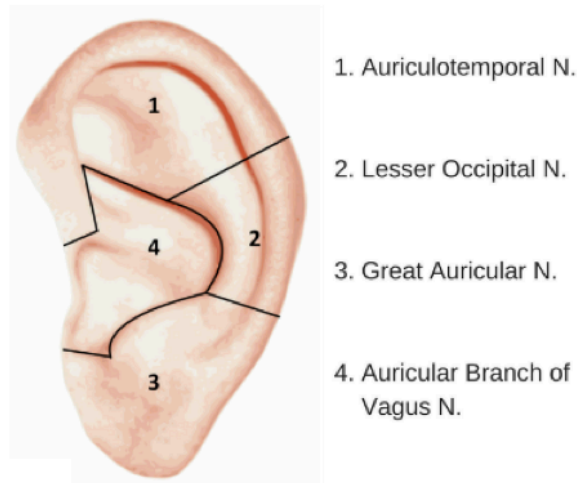


Figure 4.3: Distribution of the nerve supply of the ear.

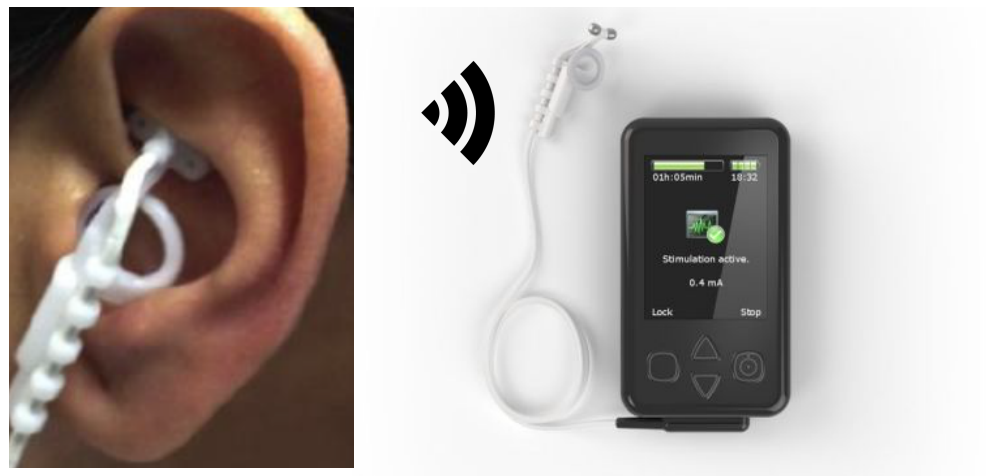


Figure 4.4: NEMOS tVNS device (www.cerbomed.com).

Study design

In view of the need for effective therapies for refractory NERD, and in view of the increasing evidence for the analgesic effects of increasing parasympathetic tone, in this study I proposed that there is value in investigating the effect of tVNS on oesophageal pain hypersensitivity in healthy volunteers. The rationale for investigating tVNS in healthy

volunteers was threefold. Firstly, studying the effect of tVNS on a validated healthy volunteer model of oesophageal pain hypersensitivity would allow us to compare the effect of tVNS with other healthy volunteer studies investigating the effect of increasing parasympathetic tone using slow deep breathing for example (12). Secondly, a cohort of healthy volunteers is likely to be more homogeneous compared to a cohort of patients with NERD, due to the well recognised heterogeneity of this group of patients. Thirdly, a cohort of healthy volunteers would afford a baseline and proof of concept for patient studies in the future.

This study was designed as a prospective randomised single blind placebo controlled crossover trial. The active intervention involved vagal nerve stimulation using the above tVNS device with the electrode stimulating the cymba conchae of the left ear (figure 4.4).

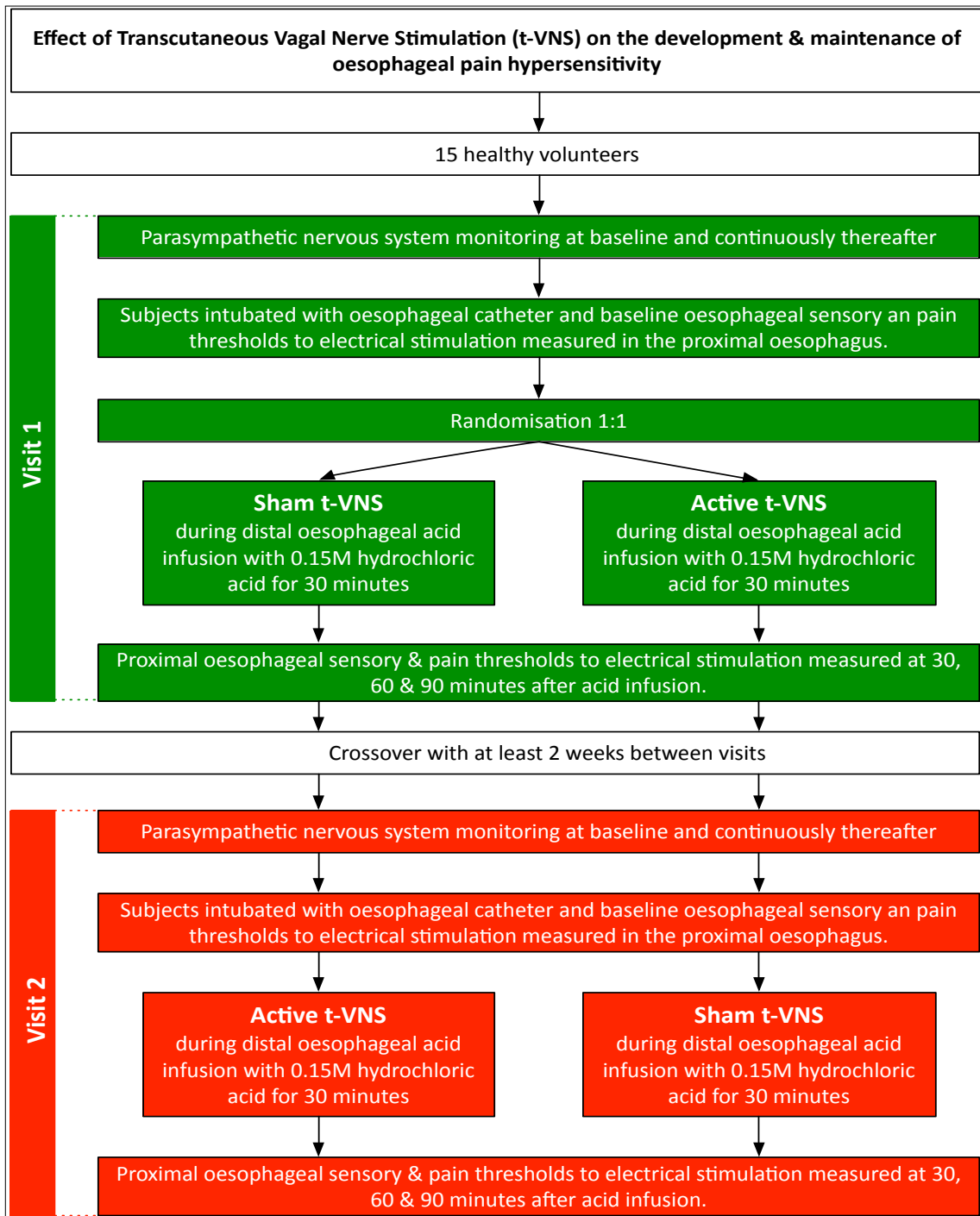


Figure 4.5: Study design flowchart.

The sham intervention involved stimulation using the tVNS device with the electrode stimulating the lobule of the ear. A sham control was chosen as a suitable control for tVNS in order to standardize the subject experience, ensuring both groups were comparable. Subjects were randomised to either start with the active intervention or the sham intervention. The study was single blinded so that the subjects were unaware of which was the active treatment. A minimum 2 week washout period was added to minimise a possible order effect.

Inclusion and exclusion criteria

Inclusion criteria:

- Healthy subjects, aged 18-65, from staff and local population of Queen Mary, University of London.
- Inclusion was determined on the basis of availability, with no prior selection bias included.

Exclusion criteria:

- Participants unable to provide informed consent.
- Participants with any systemic disease or medications that may influence the autonomic nervous system (e.g. beta-agonists or Parkinson's disease).
- Participants with a history of cardiovascular conduction problems.
- Participants who were pregnant.
- Participants who had tinnitus.
- Those with reflux disease
- Those on medication, whether prescribed or (over the counter) OTC, including acid reduction medication.

Objectives and endpoints

To determine whether electrical stimulation of the auricular branch of the vagus nerve influences the development of hypersensitivity in a validated model of acid induced esophageal pain.

Objectives

Primary objective - to compare change from baseline electrical pain tolerance threshold measured in the proximal oesophagus between the tVNS group and the sham stimulation group in response to acid infusion in the distal oesophagus.

Secondary objective - to evaluate the effect of t-VNS and sham stimulation on change in parasympathetic tone from baseline to during the acid infusion.

Endpoints

Primary endpoint – change from baseline of electrical pain tolerance threshold after acid infusion.

Secondary endpoint - the effect of t-VNS and sham stimulation on parasympathetic tone during acid infusion.

Sample size rationale and statistical analysis

Based on previous experience in our department using the oesophageal pain hypersensitivity model to study the effects of both pregabalin and deep slow breathing on oesophageal pain hypersensitivity (189) (12), our data suggested that, in order to achieve a similar difference (i.e. prevention of acid induced pain hypersensitivity) of 40% between the two groups at 5% significant level and 80% power, the minimum sample size was calculated as 15. Continuous variables were presented as mean and standard deviation, or medians and inter-quartile ranges, dependent on data distribution. Categorical variables were summarised by the mean and standard deviation.

The primary endpoint of change from baseline of electrical pain tolerance threshold after acid infusion between tvNS and sham stimulation was analysed using linear regression analyses. Comparison between tvNS and sham stimulation was done using a Wilcoxon matched-pairs test. The secondary endpoint was analysed similarly.

Analyses were performed using proprietary software (GraphPad Prism version 7.00 for MAC, GraphPad Software, La Jolla California USA, www.graphpad.com). $P < 0.05$ was adopted as the level of statistical significance.

Methods

Healthy subjects, aged 18-65, from staff and local population of Queen Mary, University of London were recruited to the study via a recruitment poster placed in staff and student areas of the University.

Potential subjects were supplied with an approved information sheet, and queries were answered prior to their first visit. The study comprised of 2 visits, spaced a minimum of 2 weeks apart. The study received ethical approval (Re: QMERC2014/56) by the Queen Mary University Research Ethics Committee (REC).

Visit 1

- Written informed consent obtained.
- A medical history was taken and inclusion/exclusion criteria were reviewed.
- If the subject was deemed eligible, they were asked to complete 3 questionnaires, to assess personality type (BFI), to assess anxiety and depression levels (HADS) and to assess anxiety state and trait (STAI state/trait).
- The subject then underwent an abbreviated HRM study to identify the upper border of the LOS.
- At baseline, and continuously thereafter, measurement of PNS tone was made using the non-invasive Neuroscope system.
- Intra-oesophageal intubation using a specialised infusion/electrical stimulation catheter was performed.
- Baseline electrical pain threshold was assessed.
- The subject then underwent a 30 minute distal oesophageal infusion of 0.15M HCL whilst undergoing tVNS or sham stimulation of the cymba conchae of the left ear.
- Electrical pain tolerance threshold was measured at 30 minutes, 60 minutes and 90 minutes following the start of the oesophageal acid infusion.

Visit 2

- Following a period of no less than two weeks, in order to reduce any potential carry over effect, participants were crossed over and re-studied to receive the intervention they did not receive in visit 1. They completed a STAI state questionnaire prior to invasive procedures at the start of visit 2.

Study questionnaires

Three questionnaires were used in the study – the big five inventory (assessing personality type), the hospital anxiety and depression scale and the state/trait anxiety assessment questionnaire. The personality type questionnaire was included as there is some evidence to suggest that some personality types may have lower baseline parasympathetic tone, with consequent higher pain tolerance thresholds (171). The anxiety and depression questionnaires were added to assess if anxiety and depression levels had any bearing on either basal cardiac vagal tone or pain tolerance threshold.

Big Five Inventory

The Big Five Inventory is a self-report inventory designed to measure the Big Five dimensions. It is a multidimensional personality inventory (44 items total), and consists of short phrases with a relatively accessible vocabulary. The validated Big Five Inventory was used to measure the personality traits of neuroticism and extroversion in particular (190).

Hospital anxiety and depression scale

The HADS is a short self-administered questionnaire that takes approximately 5 minutes to complete. It is designed to screen medical patients for anxiety and depression and is well validated (162). There are seven items on the questionnaire relating to anxiety and seven relating to depression. The subject is asked to indicate how closely they relate to

each, and a score of 0–3 is generated for each answer. An overall score (out of 21) was generated for both anxiety and depression (163).

State-Trait Anxiety Assessments

This is a widely used self-report questionnaire to assess anxiety, which has good reliability and has been validated extensively (160). As one may expect, the state questionnaire asks how the subject feels at the present moment, whereas the trait questionnaire enquires about longer term feelings of anxiety. The subject was required to select how closely they identified with 20 different emotions at that point in time on a scale of 1 (“not at all”) to 4 (“very much so”). The Trait Anxiety Questionnaire examines longer-term traits that tend towards anxiety. The subject was required to select how frequently they associated with 20 different traits on a scale of 1 (“almost never”) to 4 (“almost always”).

Study procedures

Autonomic Nervous System Monitoring

Autonomic monitoring was carried out using a Neuroscope. The Neuroscope measures real time, beat-to-beat, cardiac vagal tone (parasympathetic efferent), cardiac sensitivity to the baroreflex (parasympathetic afferent) and blood pressure (sympathetic efferent) (171).

1. The electrocardiograph was recorded by placing 3 small electrode stickers over the left and right shoulder and left side of the abdomen.
2. Blood pressure was measured non-invasively using a small sensor attached to the left index finger using a Velcro strap.

Subjects underwent baseline autonomic monitoring and measurements were taken continuously thereafter.

Acid infusion test

During this test, 0.15 M hydrochloric acid was infused into the distal oesophagus of the subject (10cm above the LOS) at a rate of 8ml/minute for a period of 30 minutes using a syringe pump device (12). The catheter used was a combined electrical stimulation and perfusion catheter (Unisensor, Gaeltec, Isle of Skye, UK).

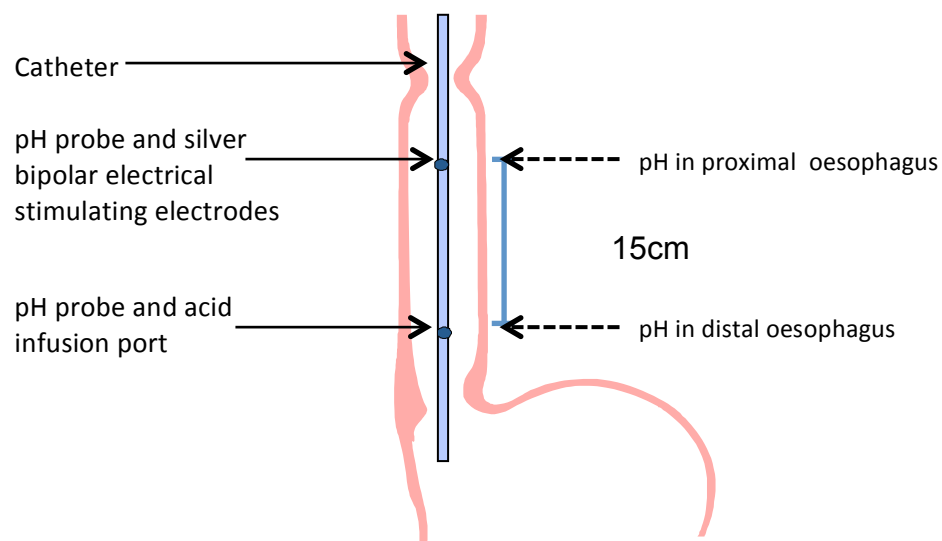


Figure 4.6: Distal oesophageal acid infusion and electrical stimulation.

tVNS and sham stimulation

The tVNS device was fitted in the active position with the stimulating electrode on the cymba conchae of the left ear, or in the sham position with the electrode on the lobule of the ear (figure 4.7). The stimulator was switched on and set to produce a pulse width of 250 μ s at 25 Hz with a 30 second “on”, 30 second “off” cycle. The intensity of the stimulus was increased from 0.1mA, in 0.1mA increments, until the participant reported a “tingling” sensation that was below the intensity that produced a noxious “pricking” sensation. The stimulation was then continued for the duration of the acid perfusion test. I was careful to attach the stimulating electrode in a similar manner, such that the subject was not able to

tell the active position from the sham position. The subject was not able to see the position of the stimulating electrode either.

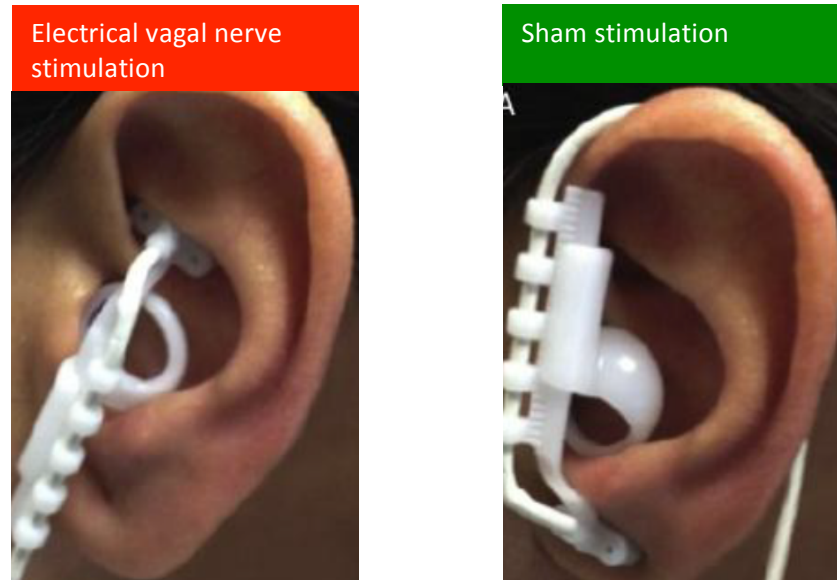


Figure 4.7: Electrode placement in the active and sham positions

Pain threshold measurements

All subjects had proximal oesophageal pain threshold measured at baseline and then at 30, 60 and 90 minutes after the acid perfusion test. This was done using a catheter with a distal infusion port and a pair of silver-silver chloride bipolar ring electrodes, although in this experiment, only the proximal electrode was used. The proximal electrode was sited 15 cm above the acid infusion port and pain threshold was determined by increasing the electrical stimulation intensity in steps of 2 mA, until the subject first reported a sensation of pain. The mean of three separate measurements was recorded as the pain threshold. The duration of the electrical stimulus pulse was 200 μ s and the frequency of stimulation was 0.5 Hz.

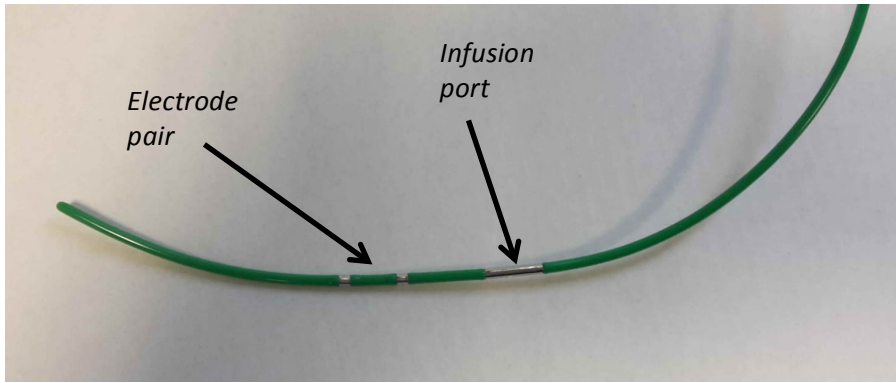


Figure 4.8: Distal stimulating electrode pair and infusion port.

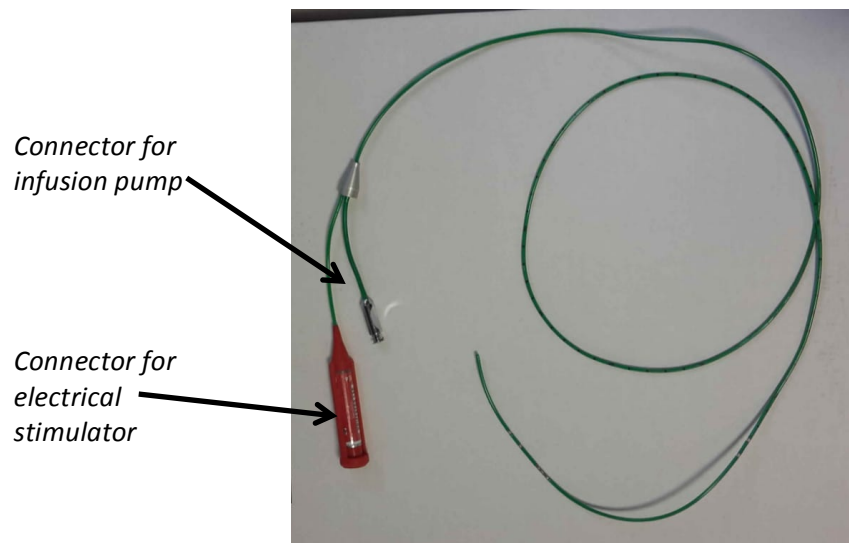


Figure 4.9: Combined electrical stimulation and perfusion catheter (Unisensor, Gaeltec, Isle of Skye, UK).



Figure 4.10: Lab set up for distal oesophageal electrical stimulation and acid infusion.

Results

The recruitment target was met with 15 healthy subjects included in the study, 6 female, with a median age of 26 years (range 21-48). Medical histories did not reveal any features to exclude any of the subjects, and in particular, migraine, epilepsy and GORD were excluded. . Median BMI was 24.60 Kg/M² (range 19.92-36.63). Subjects were Caucasian except for 2 individuals who were of Japanese and South East Asian ethnicity respectively.

Demographics						
	Gender	Age	Ethnicity	Height	Weight	BMI
1	Male	23	C	1.65	72	26.45
2	Female	25	C	1.6	51	19.92
3	Male	39	C	1.57	58	23.53
4	Male	29	C	1.62	62	23.62
5	Female	48	C	1.55	88	36.63
6	Female	22	C	1.58	54	21.63
7	Male	37	C	1.65	85	31.22
8	Female	32	C	1.68	92	32.60
9	Female	36	SEA	1.55	64	26.64
10	Male	42	JAP	1.7	59	20.42
11	Male	22	C	1.8	80	24.69
12	Male	21	C	1.84	88.4	26.11
13	Male	23	C	1.92	84	22.79
14	Male	23	C	1.75	87.4	28.54
15	Female	26	C	1.72	66.2	22.38

Table 4.1: Demographics data of subjects (C=Caucasian, SEA=South East Asian, JAP=Japanese).

Questionnaire analysis

HADS questionnaire

Mean HADS anxiety score was 4.33 ± 2.97 and mean HADS depression score was 2 ± 2.99 . The maximum score for each parameter is 21, and a score up to a value of 7 is considered normal based on validation studies. Similarly, scores from 8 to 10 are considered as borderline and scores over 11 are categorized as abnormal. One subject scored 8 and one subject scored 12 in the anxiety category, whilst only one subject scored 8 in the depression category.

STAI Trait questionnaire

This questionnaire assesses underlying anxiety traits. It was completed at baseline and the mean score was 31.4 ± 8.67 . The scores range from a minimum possible score of 20 to a maximum possible score of 80. Some of the questions are anxiety absence scores, and this is collated into a scoring algorithm to reach a final score. Cut off scores are variable between different populations, but the lower the score, the lower the anxiety levels. Therefore a mean score of 31.4 ± 8.67 denotes low levels of trait anxiety in this cohort. The minimum STAI Trait score recorded in this cohort was 20 and the maximum score was 51.

STAI State anxiety questionnaire

There was no difference in the mean state anxiety score at the start of the visit with the tVNS intervention (31.4 ± 8.67), and at the start of visit with the sham intervention (36.07 ± 12.89) ($p = 0.1836$).

BFI

BFI was assessed at the start of visit 1, before any procedures, irrespective of the nature of the treatment subsequently received in the visit. When considering the BFI at this time point, there was no correlation between personality type and *baseline* CVT for all subjects as a single group. Most subjects had dominant agreeableness scores (6/15), with fewer subjects having dominant scores for conscientiousness (2/15), extraversion (5/15) and neuroticism (2/15). Two subjects were co-dominant for agreeableness and openness.

Oesophageal pain hypersensitivity results

There was no significant drop in oesophageal pain tolerance threshold (mA) after a 30 minute acid infusion in the tVNS group at T30, T60 and T90 ($p = 0.6785, 0.9020$ and 0.7510 respectively). Therefore, mean pain tolerance threshold at baseline was 37.53mA (± 16.63), at 30 minutes was 36.09mA (± 14.87), at 60 minutes was 36.2mA (± 14.97) and at 90 minutes was 36.61mA (± 14.75). That is to say that oesophageal pain hyperalgesia was prevented in the tVNS group, and baseline pain tolerance threshold was sustained after acid infusion for a period of 90 minutes after the start of the acid infusion.

In the sham stimulation group, after a 30 minute oesophageal acid infusion, there was a significant drop in pain tolerance threshold at all three time points, T30, T60 and T90 ($0.0002, 0.0002$ and 0.0008 respectively). Therefore, mean pain tolerance threshold at baseline was 33.56mA (± 11.36), at 30 minutes was 25.22mA (± 6.888), at 60 minutes was 25.13mA (± 8.941) and at 90 minutes was 28.71mA (± 9.206). That is to say that oesophageal pain hyperalgesia was caused by the 30 minute acid infusion as expected, and sham stimulation had no effect on this (figure 4.11).

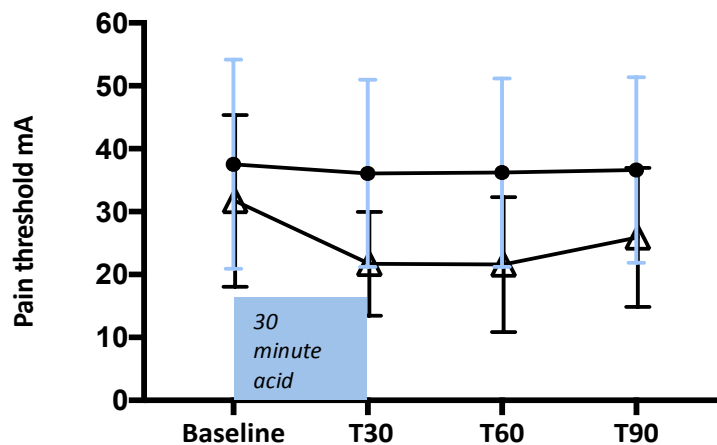


Figure 4.11: Oesophageal pain tolerance threshold (mA) for the tVNS group and the sham stimulation group. The magnitude of change in pain tolerance threshold was similar to that seen by Botha et al (176).

When change in mean pain threshold as a whole from baseline was compared, tVNS produced a significantly smaller reduction in pain tolerance threshold compared to sham stimulation ($p = 0.0155$) (figure 4.12).

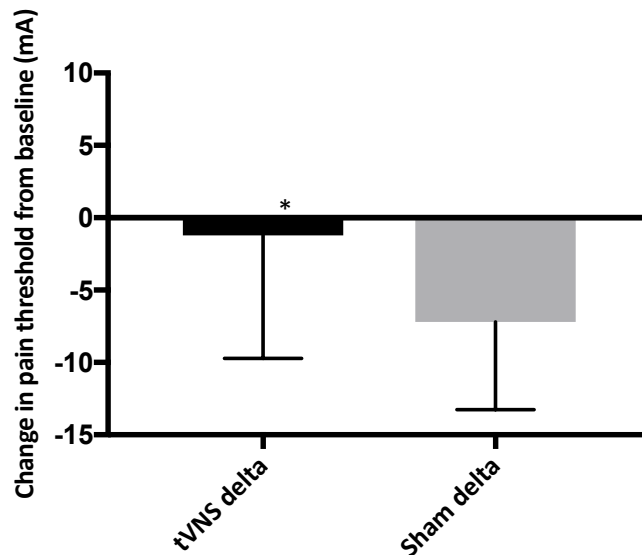


Figure 4.12: Change in oesophageal pain tolerance threshold (mA) for the tVNS group and the sham stimulation group.

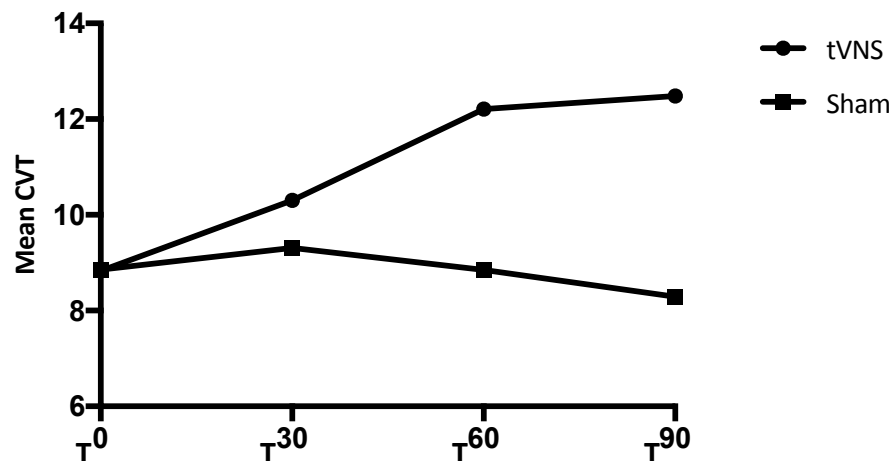


Figure 4.13: Mean CVT pain tolerance threshold at T₀, T₃₀, T₆₀ and T₉₀ for the tVNS group and the sham stimulation group, showing an increase in CVT with time in the tVNS group compared to the sham stimulation group.

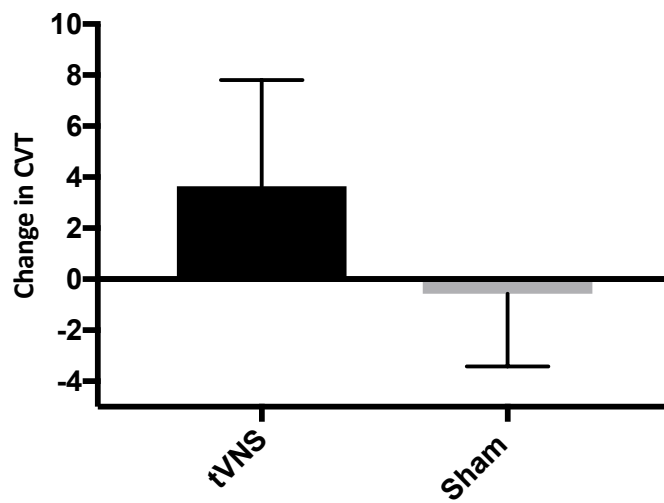


Figure 4.14: Absolute change in CVT (and thus parasympathetic tone) from baseline to T90, was significantly positive in the tVNS group compared to the sham stimulation group.

There was no significant difference between baselines CVT between either group ($p = 0.8702$). There was a significant increase from baseline in CVT in the tVNS group compared to the sham stimulation group at T90 ($p = 0.0034$).

Discussion

There is increasing evidence for the use of vagal nerve stimulation, and in particular, tVNS as a therapy for pain with studies in fibromyalgia and migraine as described previously. The mechanism of action is likely to involve modulation of parasympathetic tone, as the vagus nerve is a major component of the parasympathetic nervous system. Increasing parasympathetic tone has been shown to produce an analgesic effect (175) and therefore a means of vagal nerve stimulation is an ideal target for the treatment of pain conditions.

In this study, I have clearly shown that stimulation of the auricular branch of the vagus nerve by a non-invasive transcutaneous stimulator produced a significant increase in parasympathetic tone compared to sham stimulation. This increase in parasympathetic tone was sustained at 90 minutes post acid infusion, which is similar to results achieved with slow deep breathing in a study using the same model by Botha et al (12). There was also a sustained prevention of reduction of pain tolerance threshold in response to oesophageal acid infusion compared to sham stimulation, which again, is similar to results achieved by slow deep breathing as mentioned above. The mechanism of this sustained effect is less clear. In the short term (upto 90 minutes), it is possible that in addition to increased parasympathetic tone, changes in perception, motivation and emotion may play a part, affecting interoceptive awareness and interoceptive accuracy.

As these findings have now been shown using two differing modalities of increasing PNS tone, the role of increased parasympathetic tone in the prevention of acid induced oesophageal hyperalgesia is further confirmed. Based on this study, it is also clear that the oesophageal hypersensitivity model I used continues to perform robustly.

A limitation of this study was the absence of an arm to investigate the effects of the vagolytic drug atropine, which might have abolished the effect of tVNS, preventing the

reduction of pain tolerance threshold as was seen in the deep breathing study mentioned above.

The effect of tVNS in this healthy volunteer study paves the way for studies investigating the role of tVNS in patients with oesophageal pain, in particular, patients with NERD. This group of patients is ideally suited due to the role that increased distal oesophageal acid exposure plays in their symptomatology. Therefore, investigation of baseline and interval pain threshold levels with tVNS compared to sham stimulation is a possible means of translating the results seen in this study into the realm of therapeutic intervention, potentially adding another avenue of treatment for patients with NERD.

There is evidence to show that the therapeutic effects of vagal nerve stimulation at least in the context of depression and epilepsy is long lasting (191). The prevention of acid induced oesophageal hyperalgesia for a period of 60 minutes after the end of the acid infusion seen in this study provides evidence for longer lasting analgesic effects in oesophageal pain hypersensitivity. Dose finding work on the effect of tVNS stimulation in oesophageal pain as well as more longer term studies, initially in healthy volunteers, may help clarify the extent of this effect in the context of oesophageal pain.

Baseline PNS tone is interesting in itself, considering the multitude of ways in which the ANS contributes to the process of interoception (defined as 'the physiological sense of the condition of the body'). Awareness of the physiological state of the body is thought to be underpinned by a neural network involving afferent pathways that perceive the physiological state of the body and which project to the autonomic and homeostatic centres of the spinal cord and brain. The anterior insular cortex receives this information and forms a representation of the state of the body. This information is influenced by the anterior cingulate cortex (involved in motivation, emotion, memory and learning), and in combination, a process of interoceptive awareness occurs. Reduction in interoceptive

awareness as well as interoceptive accuracy has been associated with increased perception of pain in chronic pain conditions and pain perception (192) (118). Thus, components of this interoceptive network such as autonomic afferents may be harnessed as a means of treating pain.

An interesting extension of this work would be to extend the assessment of PNS tone in the context of ANS modulating therapies, over the longer term. Adding assessment of baseline parasympathetic tone to such work may help elucidate if any longer term anti-nociceptive effect of ANS modulating therapies is in part at least, due to an increase in baseline parasympathetic tone.

The body of work in this chapter adds to previous work demonstrating the anti-nociceptive effect of the PNS, thus paving the way forward in the search for new treatment measures for pain and disease. Further work on the role of the ANS in pain perception, inflammation, affect and health continues to be spurred on by advances in the neurophysiological basis of interoception (173).

Conclusions

The development of oesophageal hyperalgesia is prevented by transcutaneous electrical stimulation of the auricular branch of the vagus nerve. This study provides further evidence of the anti-nociceptive role of the parasympathetic nervous system. Further work is warranted in patients groups such as those with NERD and other gastro intestinal pain disorders.

Chapter 5

A randomized single blinded parallel study to investigate the effect of physiological modulation of the ANS using deep slow breathing on oesophageal pain hypersensitivity in patients with NERD

Introduction

The previous chapters in this thesis described the therapeutic gap in the treatments available for patients with refractory symptoms of GORD in the absence of macroscopic oesophageal injury (NERD). They also described the sizeable demographic that falls into this group of patients as well as the significant financial burden of refractory NERD.

Modulation of the autonomic nervous system was discussed as a therapeutic measure for the treatment of pain in the previous chapter (118) (119). Work in our group by Botha et al (176) showed that ANS modulation by slow deep breathing had an effect on pain thresholds in healthy volunteers, and in the previous chapter, I showed that tVNS did the same. This study takes this work a step further, by assessing the effects of ANS modulation in patients with NERD and oesophageal reflux hypersensitivity.

I investigated the effect of deep slow breathing compared to sham breathing on oesophageal pain hypersensitivity in a group of patients with NERD. A modified Bernstein test was used as the stimulus model in this study. This model has been used in the NERD patient population previously (152, 153), and therefore it was felt this would allow for a better comparison with similar studies. I used ANS monitoring to assess if slow deep breathing was able to increase parasympathetic tone in this population of patients with NERD.

I also investigated the effect of slow deep breathing as a self-administered potential therapeutic measure over a 4-week period.

Slow deep breathing and parasympathetic tone

The slow deep breathing protocol used was developed as a method of increasing parasympathetic tone by exaggerating the normal sinus arrhythmia controlled by the parasympathetic nervous system. This reflex is termed the Hering Breuer Reflex.

The Hering–Breuer reflex, named after the German physiologists Josef Breuer and Ewald Hering working in the 1860s, is a reflex that is activated to prevent over inflation of the lungs (193). Pulmonary stretch receptors, located in the smooth muscle of the lungs, trigger action potentials if there is excessive stretching on the airways during inspiration. Increased sensory activity of the pulmonary-stretch lung afferents (via the vagus nerve) results in inhibition of the central inspiratory drive and thus inhibition of inspiration and initiation of expiration (194). These pulmonary afferents also send projections to the cardiac vagal motor neurones in the nucleus ambiguus as well as the dorsal motor vagal nucleus, located in the brainstem. Cardiac vagal motor neurones, which provide motor fibres to the heart via the vagus nerve, are responsible for tonic inhibitory control of heart rate. Thus, an increase in pulmonary stretch receptor activity leads to activation of the cardiac vagal motor neurons, reduced inhibitory control and an elevation in heart rate. This is a normal occurrence in healthy individuals, and is referred to as respiratory sinus arrhythmia.

In an experimental setting in healthy volunteers, this reflex can be modified using deep slow breathing to physiologically elevate vagal tone. Therefore, during deep slow breathing, there is an increase in cardiac vagal tone with a concomitant decrease in heart rate, an effect that is abolished with concomitant administration of the vagolytic drug, atropine (176).

Study design

This study was designed as a randomised, sham controlled, single blinded, parallel study. A sham control was chosen as a suitable control for slow deep breathing in order to standardize the patient experience ensuring both groups of patients are comparable. Patients were randomised to either have the sham therapy or slow deep breathing. The study was single blinded so that the patients were unaware of which the active treatment was. There was no facility to blind the investigator due to a lack of personnel, although ideally this would have been preferable. A parallel design was chosen due to the fact that, as seen in the 3rd chapter of this thesis, there is a suggestion that there may be an order effect if the modified Bernstein test is repeated over more than one study period. Thus, patients were randomised to one of two groups, one receiving the active breathing protocol and one receiving the sham breathing protocol.

In contrast to the study in chapter 3 where the MBT was also used, the primary endpoint was chosen as lag time instead of APSS. As APSS is a function of lag time and sensory intensity rating, both of which are subjective stimulus responses, it was felt that using a single subjective stimulus response as an endpoint would be preferable to using a combination of two subjective stimulus responses as an endpoint.

At the end of the study period, all the patients were instructed on how to do the active breathing protocol twice a day for 10 minutes over the next 4 weeks. They were then asked to complete a symptom based and state based questionnaire after the 4-week period. This was in order to assess any possible benefit of self-administered deep slow breathing for all patients. The participants were randomly allocated, using randomisation software, such that equal numbers started in each of the two groups. It was planned to screen a suitable number of patients in order to randomise 68 subjects. Patients were recruited from the GI physiology Unit and the Endoscopy Unit at the Royal London Hospital.

Sample Size Calculation

The sample size calculation was based on the primary endpoint, mean lag time. Previous research (155) found a mean lag time of 136 seconds, with a standard deviation of 39 seconds. A difference in lag time between groups of 20% (equivalent to 27.2 seconds) was regarded as being of clinical importance. Using a 5% significance level and 80% power, it was calculated that 34 subjects per group (a total of 68 subjects), was required to detect a reduction of 20% in the primary outcome.

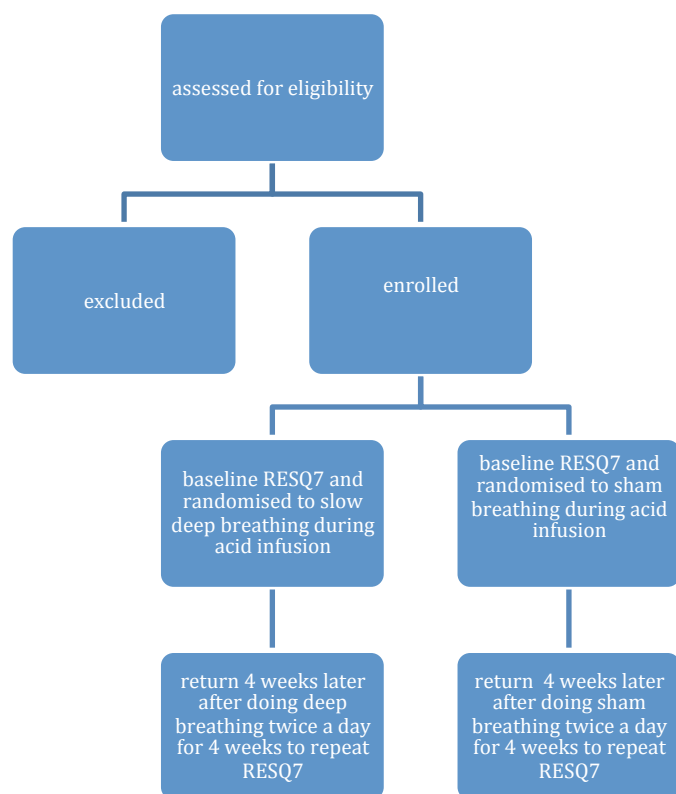


Figure 5.1: Schematic diagram of study design.

Objectives

Primary objective

To evaluate the effect of slow deep breathing and sham breathing on oesophageal pain hypersensitivity on experimental acid infusion in patients with NERD.

Secondary objectives

- A pilot follow-up study to evaluate the effect of slow deep breathing as a self-administered therapeutic measure for oesophageal symptoms in patients with NERD.
- Evaluate the effect of slow deep breathing and sham breathing following oesophageal acid perfusion on APSS (acid perfusion sensitivity score).
- Determine ANS changes before and after slow deep breathing/sham breathing.

Endpoints

Primary endpoint

- Difference in lag time to first sensation of discomfort following oesophageal acid perfusion between the slow deep breathing group and the sham breathing group.

Secondary endpoints

- Change in reflux symptom questionnaire, before and after 4 weeks, between the slow deep breathing and sham breathing groups.
- Difference in APSS (acid perfusion sensitivity score) following oesophageal acid perfusion between the slow deep breathing group and the sham breathing group.
- Comparison of ANS changes before and after slow deep breathing versus sham breathing in visit 1.

Methods

Duration of patient participation and visit details

Patients were enrolled for a period of 4 weeks, and attended the Wingate Institute for 2 visits. *The first visit* consisted of informed consent and checking for eligibility, after which the patients were randomised to start with either the slow deep breathing protocol or the sham breathing protocol in a single blinded fashion. That is, the patient was unaware of whether they were receiving the active breathing or the sham breathing exercise. Once randomized, they completed a baseline RESQ 7 questionnaire after which they underwent an acid perfusion test whilst performing the relevant breathing exercise. The patient was then trained to self-administer the breathing exercise used at visit 1, to be used twice a day for 10 minutes over the next 4 weeks.

Visit 2 occurred 4 weeks later when they attended the Wingate Institute in order to complete another RESQ 7 questionnaire, demonstrate their breathing exercise technique, and to debrief.

Visit 1

- Written informed consent
- Demographics, medical history and medication history
- Questionnaire (RESQ-7)
- Physical examination, vital signs and ECG
- Inclusion / exclusion criteria review
- Randomisation
- Baseline autonomic nervous system (ANS) recording 10 minutes, and continuously thereafter.
- Intubation with oesophageal catheter with 10 minutes to accommodate.
- Start breathing protocol 5 minutes before start of Modified Bernstein test.
- Modified Bernstein test (2 minutes saline followed by 10 minutes 0.1M HCL).

Visit 2

- Complete RESQ 7 questionnaire.
- Demonstrate breathing exercise technique to ensure good practice.
- Debrief session to review compliance, ease of use and any issues encountered.

Eligibility criteria

Inclusion criteria

- Male and female patients over the age of 18 years.
- Women in the follicular phase of the menstrual cycle (visits will be arranged so that in menstruating women, the study will be started in the follicular phase of the menstrual cycle to standardise for possible confounding effects of the menstrual cycle on symptom perception).
- Able to give informed consent
- Able to speak and understand English without the need for an interpreter
- Negative OGD
- Positive reflux study (a reflux study confirming GORD or RH)
- An established diagnosis of NERD

Exclusion criteria

- Current or previous GI or medical illnesses that may affect ANS / GI function
- Current or previous significant CNS illness
- Current medications affecting the CNS, GI or ANS systems
- Scoring less than very mild at the modified Bernstein test
- Pregnancy and lactation
- Cardiac dysrhythmias

Patients were asked to refrain from smoking for 12 hours and drinking alcohol as well as using recreational drugs for 48 hours prior to study visit.

Deep breathing protocol

The deep breathing protocol used in this study consisted of a cycle of deep breathing at full inspiratory capacity for 4 seconds followed by forced expiration in 6 seconds (forced vital capacity), at a frequency of 6 breaths per minute for every 5th minute for 5 minutes prior to and for the duration of a modified Bernstein test (described in previous chapters), which involves infusion of acid into the lower oesophagus and is validated for assessment of acid induced oesophageal hypersensitivity in GORD patients. A standard video providing a demonstration of slow deep breathing and sham breathing was used in order to ensure standardisation and consistency in the advice given to patients about the slow deep breathing versus sham breathing protocol, which was of paramount importance. The surroundings were kept neutral and relaxing with minimal distractions.

Sham breathing protocol

The sham breathing protocol was developed using breathing as a basis, but with no particular instruction regarding depth or tempo of breathing. Patients were instructed to count 10 breaths and tick a box every time they counted ten breaths. The counting distracts the patient reducing the effect of focussing of breathing on their autonomic nervous system (12). The surroundings were kept neutral and relaxing with minimal distractions.

Study procedures

Autonomic Nervous System Monitoring

Autonomic testing was carried out using a Neuroscope. The Neuroscope measures real time beat-to-beat cardiac vagal tone (parasympathetic efferent), cardiac sensitivity to the baroreflex (parasympathetic afferent) and blood pressure (sympathetic efferent).

Modified Bernstein Test

During this test, 0.9% saline was initially infused into the oesophagus (10 cm above the lower oesophageal sphincter) at a rate of 10 mL/min for 2 minutes. Subsequently, without the subject's knowledge, 0.1 M hydrochloric acid was infused for 10 minutes at the same rate.

Stimulus-response functions to acid were quantified by lag time to symptom perception, Sensory intensity rating (at the end of the acid perfusion); and APSS (acid perfusion sensitivity score).

Lag time was defined as the time (in seconds) to initial first symptom perception. Lag time values for healthy controls have been previously assessed by Fass et al (152). An assessment of the intensity of symptoms associated with acid perfusion was made using a verbal descriptor scale (154). The scale consists of a 20-cm vertical bar flanked by descriptors of increasing intensity (no sensation, very weak, faint, weak, very mild, mild, moderate, barely strong, slightly intense, strong, intense, very intense, and extremely intense). Placement of words along the scale was determined from their relative log intensity rating in a normative study (156). The validity of these scales for assessing the perceived intensity of visceral sensation has been confirmed (157).

Deep breathing protocol and sham breathing protocol administration

During both breathing protocols, the patient watched and following a standardised instructional video in a quiet and calm environment for the duration of the Modified Bernstein test.

Questionnaires

- **RESQ – 7**

This is a well-validated questionnaire used in patients with GORD who experience only a partial response to PPI therapy. It is brief and easy to complete and is intended for use in routine clinical care. It consists of 13 items incorporating oesophageal and extra oesophageal symptoms of GORD, and requires the patient to document frequency and intensity of symptoms over the previous 7 days. It is frequently used in clinical trials assessing new therapies for the treatment of GORD and it would therefore be a suitable questionnaire to study the effect of treatment on symptoms of GORD (159). It was also the questionnaire used to assess the value of self-administered slow deep breathing at the end of the study.

Data analysis and statistical considerations

Outcome measures

As mentioned above, stimulus–response outcome measures to acid perfusion were quantified using three standard validated parameters:

- Lag time: Defined as the time to initial symptom perception following acid perfusion.
- Sensory intensity rating: An assessment of the intensity of symptoms at the end of the acid perfusion made using a previously validated verbal descriptor scale at the end of the acid perfusion
- APSS: calculated from lag time (T) expressed in seconds (sec) and sensory intensity rating (I) expressed in centimetres (cm).

Although lag time was used as the primary endpoint, studies using the Modified Bernstein test often use the APSS, and so for the sake of comparison, I calculated this as well (47). The APSS was calculated by multiplying the lag time to symptom perception (expressed in seconds) and the symptom intensity rating (expressed in cm on a validated scale), divided by 100 for convenience to yield a score in cm seconds.

$$APSS = (I) \times (T)/100 \text{ (cm} \times \text{sec}/100\text{)}.$$

Statistical Analyses

Continuous variables are presented as mean and standard deviation, or medians and inter-quartile ranges, dependent on data distribution. Categorical variables were summarized by the mean and standard deviation.

The primary endpoint, lag time (in seconds) to first sensation of discomfort following oesophageal acid infusion was analyzed using the Mann-Whitney test because of the assumed non-parametric nature of the data. Lag time data was analysed for normality using the Shapiro Wilk normality test and only the slow deep breathing group passed the normality test. RESQ7 scores were analysed using the methods as described for the primary outcome.

Analyses were performed using proprietary software (GraphPad Prism version 7.00 for MAC, GraphPad Software, La Jolla California USA, www.graphpad.com). $P < 0.05$ was adopted as the level of statistical significance.

Results

116 patients who were eligible to participate were approached. They were provided with an information sheet. 35 patients agreed to participate in the study and attended for visit 1. Of these 35, 5 met exclusion criteria and did not continue with visit 1. Therefore 30 patients were randomised and completed visit 1 of the study. There were no drop-outs between randomisation and completion of visit 1. 24 of the 30 patients completed visit 2 of the study, although all 30 were given the opportunity to participate.

The demographics of patients approached who declined to participate were not collected. In general however, the reason for declining was informally noted, with reluctance to undergo further non-essential invasive tests (after having completed their clinically indicated HRM and 24 hour pH studies) being the predominantly cited reason.

There were equal numbers in both arms of the study, and both groups were well matched for age and sex. As a whole, median age of the 30 participants was 55 (44 – 60). 11 were female. 23 were of white ethnicity, with 4 of south Asian ethnicity, 2 of black ethnicity and one other. Median BMI was 28.21 Kg/m² (25.15 – 32.22).

All participants had a diagnosis of NERD, with a confirmed negative gastroscopy and 24 hour pH or MII pH study. 25 were on a regular dose of a PPI, with the other 5 using an alginate (Gaviscon[®]) as required. 1 participant was on low dose amitriptyline and one was on treatment dose amitriptyline (for a diagnosis of depression), 2 were on an SSRI (one for a diagnosis of depression) whilst one was on an SNRI (for a diagnosis of depression).

Lag time analysis

Analysis of lag time formed the primary endpoint of the study. Mean lag time for the slow deep breathing group was 203.9 seconds (± 140.9) and median lag time was 168 (102-240). For the sham breathing group, mean lag time was 231.8 seconds (± 166.2) and median

lag time was 185 seconds (143-319). There was no significant difference between the two groups when comparing lag times ($p = 0.6022$). The difference in APSS was not significant either ($p = 0.8702$).

Lag time values for the slow deep breathing group were more evenly scattered, compared to the sham breathing group, where there was a notable outlier. Although removal of this outlier from the data set altered the mean and median lag time values in the sham breathing group, when comparing both groups, there was still no significant difference between both groups ($p = 0.8218$).

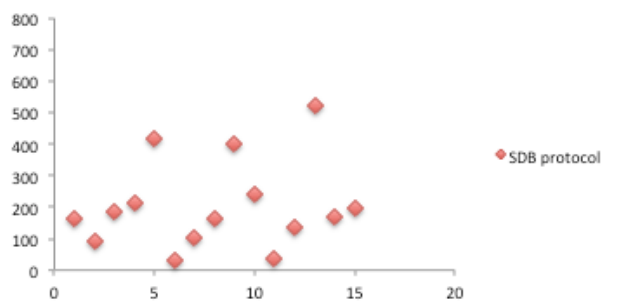


Figure 5.2: Distribution of lag time for the slow deep breathing group.

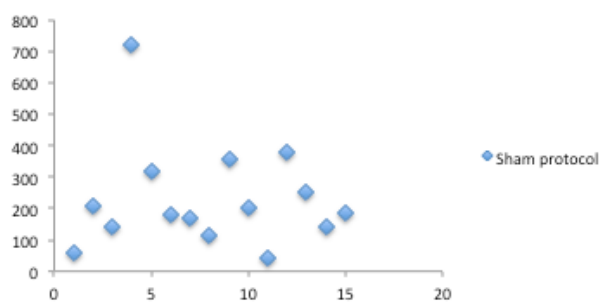


Figure 5.3: Distribution of lag time for the sham breathing group.

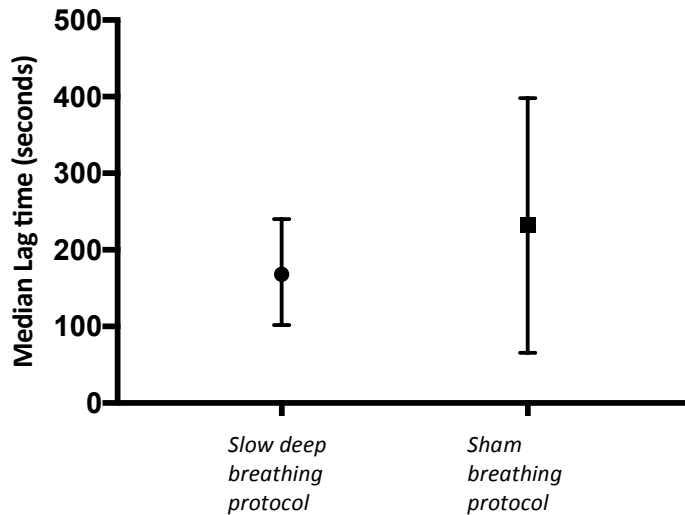


Figure 5.4: There was no significant difference between the two groups when comparing median lag times ($p = 0.6022$).

Cardiac vagal tone analysis

Comparing ANS changes before and after the two breathing protocols was a secondary endpoint of the study. Mean baseline CVT was 4.333 (± 2.514) for the slow deep breathing group and 5.659 (± 2.555) for the sham breathing group. There was no significant difference between the 2 treatment groups ($p = 0.1488$).

Pre Bernstein mean CVT during the deep breathing protocol was 5.751 (± 3.576), and during the sham breathing protocol was 5.944 (± 2.933). During the Bernstein test, mean CVT during the deep breathing protocol was 6.063 (± 3.714), and during the sham breathing protocol was 5.809 (± 3.037).

The mean change of CVT from baseline to pre Bernstein breathing protocol was 1.42 for the deep breathing group and 0.28 for the sham breathing group. The mean change of CVT from baseline to the Bernstein plus breathing protocol was 1.7 for the slow deep

breathing group and 0.15 for the sham breathing group. These changes were of the same magnitude as the study by Botha et al (176).

There was a significant rise from baseline CVT when participants performed slow deep breathing ($p = 0.0052$). The rise from baseline remained significant when the participants performed slow deep breathing during the Modified Bernstein test as well ($p = 0.0052$).

In the sham breathing group however, this was not apparent. There was no significant rise in CVT from baseline when the participants performed sham breathing ($p = 0.5245$). CVT did not rise from baseline when the participants performed sham breathing during the Modified Bernstein test either ($p = 0.7197$).

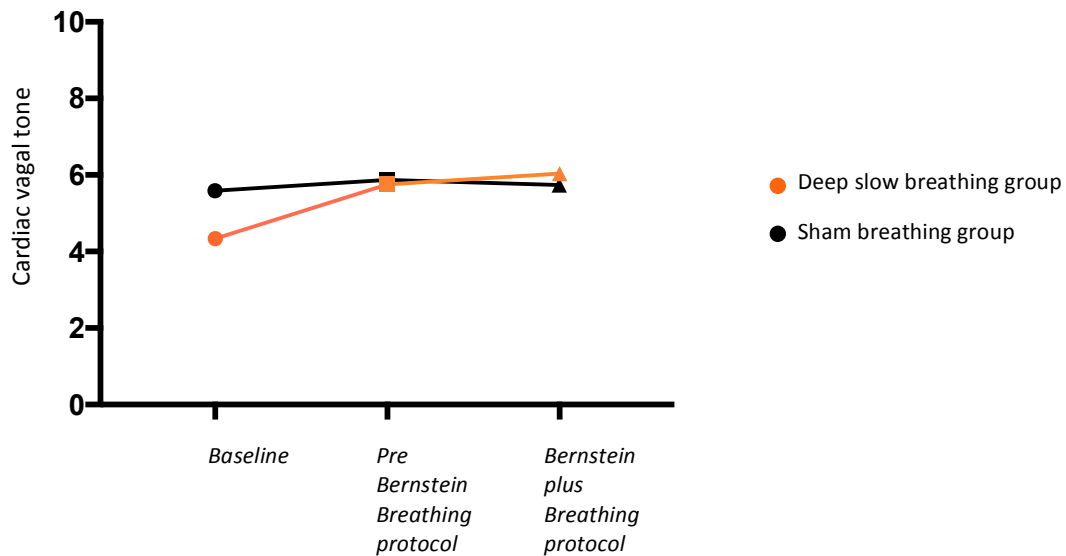


Figure 5.5: There was a significant rise from mean baseline CVT in the deep slow breathing group with deep slow breathing alone ($p = 0.0052$) as well as with deep slow breathing during a Modified Bernstein test ($p = 0.0052$). This was not shown in the sham breathing group ($p = 0.5245$ and $p = 0.7197$) respectively.

Questionnaire analysis

Questionnaire analysis was done prior to any procedures and served a baseline for both groups. Mean baseline RESQ7 score was 62.73 (± 28.55) for the slow deep breathing group and 51.57 (± 28.63) for the sham breathing group. There was no significant difference between mean baseline RESQ7 for the 2 groups ($p = 0.3823$).

After 4 weeks of twice daily breathing exercises, mean RESQ7 score for the slow deep breathing group was 53.23 (± 32.67) and mean RESQ7 for the sham breathing group was 43.27 (± 26.85). There was no significant difference from baseline to follow up scores in either group (figure 5.4).

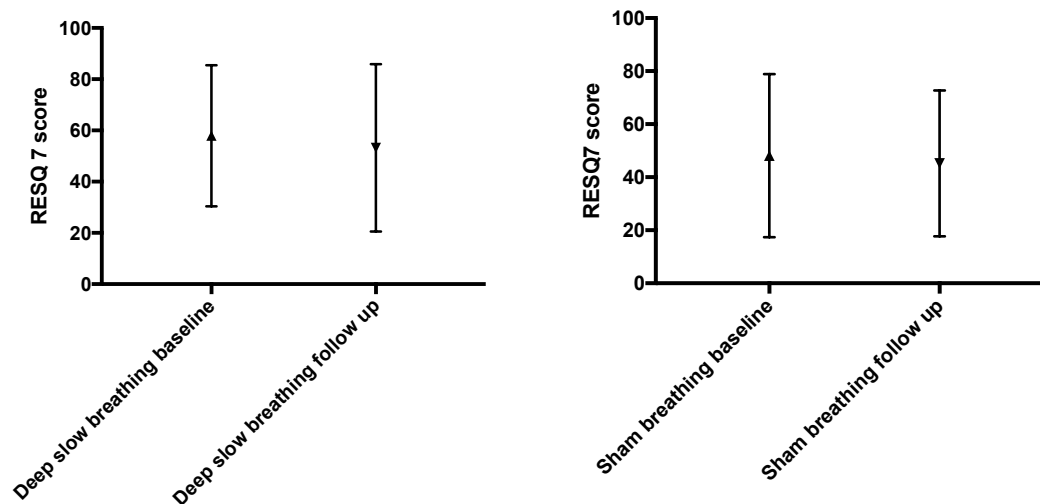


Figure 5.6: There was no significant difference between the baseline vs follow up scores for either the slow deep breathing group ($p = 0.2354$) or the sham breathing group ($p = 0.8867$).

Comparison of RESQ7 scores was made between visit 1 and visit 2 for each group. Thus, the change from baseline scores for each subject was expressed as a delta value for both groups. I then compared mean delta values between the slow deep breathing group and the sham

breathing group. There was no significant difference between the delta RESQ7 scores between the slow deep breathing group and the sham breathing group. That is to say, the mean change from baseline RESQ7 score between the 2 groups was not significant ($p = 0.6150$) (figure 5.5).

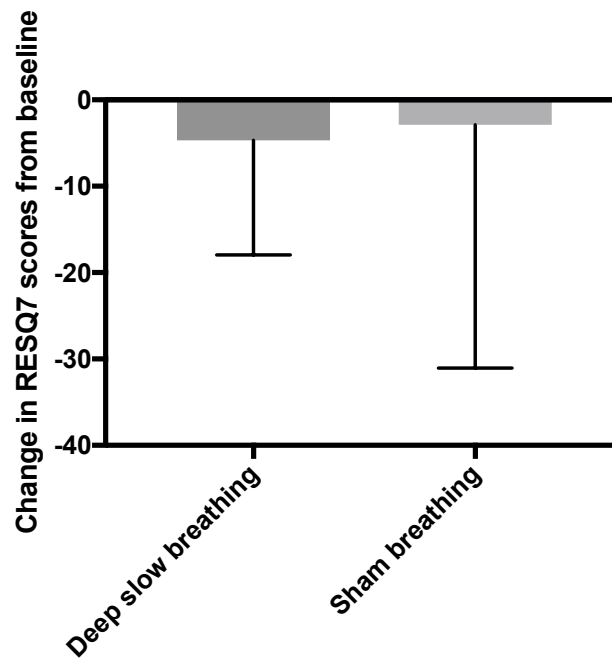


Figure 5.7: Mean change from baseline RESQ7 score to follow up RESQ7 score was not significant between the 2 groups ($p = 0.6150$).

Summary

In this study I have shown that a slow deep breathing protocol produced a significant rise in parasympathetic tone from baseline when compared to a sham breathing protocol.

I was not able to show a significant difference in lag time, defined as the time to initial symptom perception following acid perfusion, when I compared the slow deep breathing protocol group to the sham breathing protocol group. APSS was not significantly different either.

Both groups were equally matched with regards to symptom scores. These scores at baseline did not significantly change after 4 weeks of self-administered slow deep breathing.

Discussion

The study in this chapter set out to investigate if a deep slow breathing protocol was able to raise parasympathetic tone, and thus reduce oesophageal pain hypersensitivity, in comparison to sham breathing.

The power calculation used in this study was aimed at detecting a difference in lag time of 20% between the 2 groups. Using a 5% significance level and 80% power, it was calculated that I required 34 patients in each group to detect a reduction of 20% in the primary outcome. The sample size was not achieved due to difficulties in recruitment. As mentioned above, 116 eligible patients were approached, with a yield of 30 patients completing visit 1. If I revised down my aim, to detect an even smaller difference of 10% between the 2 groups, I would have met our sample size. I still would not have achieved a positive result, as the difference in lag time between the 2 groups was not significantly different.

The low uptake from multiple recruitment strategies is not a new phenomenon in the field of GORD research. As described in chapter 3, in the ONO 8539 study, there were also significant recruitment difficulties encountered. It is possible that although refractory GORD symptoms are problematic for a significant proportion of patients, current experimental models are relatively invasive and perhaps less appealing from a patient's perspective.

As mentioned above, I was able to show that a slow deep breathing protocol produced a significant rise in parasympathetic tone from baseline when compared to a sham breathing protocol. This rise was similar to the study in healthy volunteers by Botha et al (176). Here we may also ponder the potential mode of action of pain reduction by means of deep slow breathing as an effect of increasing interoceptive accuracy by raising parasympathetic tone. As mentioned previously, an increase in interoceptive accuracy has been shown to be associated with reduced pain and symptom severity, so this is a plausible idea.

It is unclear why a significant rise in parasympathetic tone, which in previous studies has been shown to increase oesophageal pain threshold, did not lead to a change in lag time to initial symptom perception following acid perfusion. The cohort of patients studied were phenotyped as carefully as possible, considering previous pitfalls described in the literature (70, 105). Another possibility is the fact that I used a primary endpoint that is perhaps more susceptible to subjectivity than an alternative endpoint such as pain threshold to electrical stimulation, which is more definitive and likely to give a more objective endpoint (103). Efforts to hone an experimental model for NERD patients with more objective endpoints would be helpful in the search for newer therapies in the field of oesophageal pain hypersensitivity.

A further reason for a lack of an increase in lag time might have been because the dose of the slow deep breathing protocol in my study may have been inadequate. Botha et al (176) used a 35 minute slow deep breathing protocol (30 minutes of which were during a distal oesophageal acid infusion), whilst I used 17 minutes, 5 of which were prior to the start of the modified Bernstein test. Further work with dose finding experiments may help in this instance. It is possible that I did not see a difference in lag time because both breathing protocols behaved as a distraction technique. Distraction and attention have been shown to reduce pain perception (195) (196). In the model that was used in this study, there was no baseline for the response to acid infusion, hence the inability to assess if there was a difference in one or both of the protocols from baseline. I consider this to be a limitation of this study.

Based on the above, an updated model of oesophageal pain hypersensitivity is needed for experimental work in patients with NERD. The 30 minute oesophageal pain hypersensitivity model described in chapter 4 as well as in work by Botha et al and Sarkar et al has been used in studies involving patients with NERD (197) but based on experience in our group, is likely to be less tolerated in patients with NERD due to possible underlying peripheral as well as central sensitization. In addition to this, as mentioned previously, recruitment difficulties are likely to be increased with more prolonged invasive testing. I propose therefore that a similar testing protocol using pain threshold tolerance as an endpoint but with a shorter period of acid perfusion (shorter than 35 minutes) is likely to be better tolerated.

The secondary endpoint of this study was to assess change in reflux symptom scores between before and after 4 weeks of using slow deep breathing or sham breathing as a self-administered therapeutic measure for oesophageal pain in patients with NERD. This was not shown in my study. Although I did collect self reported compliance data suggesting good

compliance overall, this is likely to be open to error, which was a limitation of this study. Furthermore, I did not reach my intended sample size and it is therefore possible that the study was underpowered to assess any change in symptoms. Finally, a dose finding study of frequency and duration of slow deep breathing is required to determine the optimal parameters for this intervention.

In conclusion, slow deep breathing has been shown in this study to increase parasympathetic tone compared to sham breathing. Further work is required to elucidate the lack of reduction in time to perception of symptoms, including improving my current model of assessing changes in oesophageal pain hypersensitivity in response to therapeutic measures.

Chapter 6

Discussion

GORD is a leading cause of gastrointestinal morbidity world wide, with a significant financial burden (37). With rising rates of obesity, the incidence of GORD is predicted to increase further still (198). Although lifestyle modifications (199) and PPI therapy form the mainstay of treatment in GORD, we have little else to fall back on when they are ineffective. Alginates, H2RAs as well as isomeric PPIs, as well as combinations of these drugs are the current add on therapies available for refractory GORD whilst surgical and endoscopic therapies serve as means of primarily augmenting the GOJ to improve extent of reflux (200). In addition to this, considerable efforts have been made to improve diagnostic algorithms in GORD, in order to ensure we optimize current available therapies (201).

Oesophageal RH addressed in previous chapters of this thesis is thought to arise from physiological distal oesophageal acid exposure as well as non-acid exposure. The mechanism of oesophageal pain here is thought to involve peripheral sensitization from exposure of nociceptors to acid and non-acid reflux possibly facilitated via dilated inter cellular spaces in addition to central sensitization of spinal dorsal horn neurons (94). The placement of RH in the category of a functional type disorder in ROME IV is therefore an attempt at facilitating strategies for studies into new therapies for RH (2).

Although newly re-defined, RH is not a new pathology. Despite this, the epidemiology of RH is not well defined and nor is the phenotype. In this thesis, I have added

to the understanding of the phenotype of the patient with RH. I have confirmed that RH shares phenotypical attributes with both NERD and FH/ FCP. Crucially though, in chapter 2, I demonstrated that our cohort of patients with RH lie more towards the NERD end of the spectrum than the functional end of the spectrum. Considering this is the largest cohort of patients with RH studied at present, these findings may have a bearing on the future of where this condition sits best from a mechanistic point of view.

Recent work by Frazzoni et al have shown impaired mucosal clearance and reduced mean baseline nocturnal impedance (MBNI) of the oesophagus in patients with RH compared to FH (133), a finding seen in other studies as well (202). Woodland et al demonstrated that the distribution of afferent nerves in the oesophageal mucosa is more superficial in NERD compared to FH (203), reinforcing the idea that symptoms in NERD may be due to exposure of afferent nerve endings to noxious stimuli via impaired mucosal integrity in the form of dilated intercellular spaces, a surrogate marker of which is reduced mean baseline impedance (204). In view of reduced MBNI levels seen in RH, this suggests that the observations of Woodland et al are likely to be present in RH as well. It would therefore be enlightening to assess MBNI in our cohort of patients with RH as well. This will also add weight to the search for topical mucosal protectants as a therapeutic measure as shown by Woodland et al (205).

In terms of other treatment options, in chapter 3, I assessed an EP1 receptor antagonist. This was an exploratory study, and unfortunately the sample size was not achieved. The sample size calculation was based on pre clinical studies, and due to the lack of data on the effect of ONO-8539 on oesophageal pain, formal power calculations were not possible. It is not possible to know whether I might have seen an increase in lag time or APSS

even if I achieved the sample size of 30. There is also the issue I discussed in chapter 5, regarding the possibly subjective nature of lag time as a stimulus response measure. To counter this, electrical pain threshold was also measured in this study but a significant difference was not seen between ONO-8539 and placebo. Further, well-designed exploratory studies may help in this respect. The fact that antagonism of other promising receptor targets such as the TRPV1 have not been shown to reduce oesophageal pain (100) despite valiant efforts should not, however, deter the search for new targets. In contrast, in healthy subjects, oesophageal pain hypersensitivity to electrical stimulation following acid infusion has been an effective model to demonstrate efficacy of pharmacological (pregabalin) and non-pharmacological (slow deep breathing) therapies. Therefore, in future studies, sensitivity to electrical stimulation, or reduction in symptoms may be more effective end points in well-powered studies.

My studies have highlighted a possible limitation of this modified Bernstein test (MBT) model, because of the order effect seen when multiple MBTs were carried out. This was apparent despite a 4 week period between each MBT. It is possible that the order effect seen was more due to reduced anxiety levels when faced with subsequent MBTs but I was not able to show this in my analysis of change in anxiety scores. The attention received from, and inevitable rapport developed with the study team, during a 10 visit clinical trial, may have had an effect as well, which was not controlled for.

Learning effects are not uncommon in cross over experimental studies, and more complex invasive measures are prone to the occurrence of an order effect due to this. In animal models this is well recognized with the use of multiple test batteries, and caution is exercised to reduce the bias this brings to experimental work (206). The situation appears

more complex in humans, and experimental models have shown that the brain is able to rapidly adapt and learn, with this manifesting as a carry over effect from a preceding stimulus or event (207).

To counter this effect, I designed the study in chapter 5 as a parallel study, so that a carry over bias was not possible between the slow deep breathing group and the sham breathing group. The study was also of a shorter duration, in an attempt to reduce potential effects from prolonged contact with a study team. A parallel study such as this has the disadvantage of requiring a larger sample size to demonstrate any meaningful difference between treatment arms. I was not able to achieve my sample size however, which highlights again, the problem of recruitment in this patient population.

I have discussed the fact that the APSS may have limited use in the evaluation of patients when investigating therapies for oesophageal pain. There is a paucity of use of the APSS in patient studies of therapies for oesophageal pain, and the subjective nature of the components of the APSS is less preferable in the context of such studies. A single subjective measure such as lag time may have been appropriate, but as discussed in chapter 5, the use of lag time as an endpoint is also likely to be problematic, due to the subjective nature of the request being posed to the study subject. A perception of “slightest discomfort” is less easy to define than a perception of pain tolerance, supporting the idea of a need to update this model for oesophageal pain in patients with NERD.

In chapter 4, I investigated the effect in healthy volunteers, of tVNS versus sham stimulation of the auricular branch of the left vagus nerve using an oesophageal pain

hypersensitivity model. As described in chapter 4, this model had been used extensively in healthy volunteer studies with good reliability and reproducibility. Based on the work in this thesis, it appears that the above reliability and reproducibility of this model is probably due to the use of more objective stimulus response measures. An order effect was not seen in this study, nor has it been observed in previous studies using this model. Therefore, the learning effect did not seem to play a part here, even in the context of a shorter 2 week washout period. This lends weight to the attention and rapport aspect of the study in chapter 3 as a stronger contender for the cause of the order effect. It should be noted however, that even if this was the case, although there may have been a smaller learning effect between the first and second MBTs which I did not detect, the effect of 2 previous MBTs on a third and fourth MBT, 4 and 8 weeks later is likely to present a much stronger learning stimulus.

A rise in parasympathetic tone was observed in the treatment groups of both studies where ANS monitoring was used. In chapter 4, I was also able to demonstrate a rise in stimulus response, whilst in chapter 5, I was not (in terms of pain tolerance and lag time respectively). The lack of a stimulus response in chapter 5, as discussed above may be due to limitation of the model used. Either way, the work in this thesis demonstrated a rise in parasympathetic tone in the treatment group as a consistent finding. This reinforces the idea that the treatment modalities used (tVNS and deep slow breathing) were both able to significantly increase parasympathetic tone compared to sham. A proposed mechanism for a possible reduction of pain in this instance was discussed in the context of improved interoceptive accuracy by increasing parasympathetic tone (modulating the autonomic afferent input).

In conclusion, based on the above discussion, I suggest that we need for a newer model of oesophageal pain hypersensitivity in NERD patients, in order to evaluate novel therapies for oesophageal pain as well as to further elucidate the mechanistic aspects of how these novel therapies may help. Based on the work in chapter 4 and 5, the need for ANS testing, even in treatment strategies to increase parasympathetic tone as an anti-nociceptive measure, appears less crucial as there is now significant evidence that these measures are indeed able to increase parasympathetic tone. During the development stage of a new model, confirming consistent ANS changes was however needed for validation purposes.

Further work

A new model of oesophageal pain hypersensitivity in NERD patients as discussed above is likely to involve the following –

- a shorter duration of distal oesophageal acid infusion (that is, 30 minutes or less),
- a more objective stimulus response (pain tolerance threshold),
- a 4 week washout period (in view of the fact that an order effect was not seen in the healthy volunteer model),
- a cross over study (in view of recruitment difficulties in this study population),
- ANS monitoring at baseline and extended time points to measure the durability of the stimulus response.
- Addition of 0.9% saline as a null stimulus may be an idea to assess as well.

In addition to this, in view of current MNBI findings, I could consider assessing the longer term benefit of treatment strategies, including those designed to increase parasympathetic tone, using MNBI as a marker of response, considering the clear differences have been reported between RH and FH as well as before and after PPI therapy (133, 204). The fact that diagnostic algorithms especially in refractory GORD include MII-pH monitoring, this has the added advantage of a ready made baseline value if the therapeutic intervention studies are timed appropriately, to reduce research related additional invasive procedures.

Alternatives to ANS monitoring using the Neuroscope device will also help move forward future work on the investigation of ANS modulation as a therapy for oesophageal pain. This is because the neuroscope technique is expensive and not very portable. Newer, cheaper and more portable ambulatory methods are now available

that may facilitate future studies especially where long-term ANS measurements are required.

Additionally, to complement my findings in chapter 2, confirmation of afferent nerve distribution in RH compared to NERD and FH may help increase our understanding of the mechanism of pain hypersensitivity in RH.

In summary, in this thesis, this body of work improves upon current knowledge of the phenotypic characteristics of RH, adding further weight to the definition of RH as a distinct condition. I have demonstrated that tVNS and deep slow breathing increase parasympathetic tone in healthy volunteers and patients with NERD. I was able to demonstrate the anti nociceptive effect of raising parasympathetic tone a healthy volunteer model of oesophageal pain hypersensitivity, but not in patients with NERD using the MBT model. The performance of the MBT model used in the two patient studies was not as reliable as the healthy volunteer model, and I have proposed a new oesophageal pain hypersensitivity model for patients with NERD.

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