

INEFFECTIVE OESOPHAGEAL MOTILITY IN PATIENTS WITH DYSPHAGIA AND GASTRO-OESOPHAGEAL REFLUX DISEASE

Jafari, Jafar

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**INEFFECTIVE OESOPHAGEAL MOTILITY IN PATIENTS
WITH DYSPHAGIA AND GASTRO-OESOPHAGEAL
REFLUX DISEASE**

PHYSIOPATHOLOGY, DIAGNOSIS AND TREATMENT

**A thesis submitted for the degree of
Doctor of Philosophy**

Jafar Jafari

**The Wingate Institute of Neurogastroenterology
Barts and the London School of Medicine and Dentistry
Queen Mary University of London, UK**

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Abstract

Oesophageal hypomotility is prevalent in 30-50% of the patients with GORD and/or dysphagia. Despite advances in diagnosing oesophageal hypomotility, there is no established therapy for this group of patients.

I studied the effect of Azithromycin in patients with Ineffective oesophageal motility (IOM). I assessed the value of stimulation tests during oesophageal manometry (multiple rapid swallows, bread swallows and swallows with abdominal compression), in identifying the patients who might benefit from prokinetic treatment with Azithromycin.

Effect of stimulation tests in healthy subjects was investigated and normal ranges for oesophageal response to these tests was established. Characteristics of normal proximal oesophageal motility were defined and the role of proximal oesophageal hypomotility in symptomatology of the patients with IOM investigated. Effect of azithromycin on IOM and on the symptoms of these patients were studied in a double blind placebo controlled parallel design study. The predictive value of the stimulation tests in identifying the responders to azithromycin therapy was evaluated.

Stimulation tests proved to be effective on inducing stronger motility response in oesophageal body and this effect was reproducible. Weak proximal oesophageal motility in patients with IOM is associated with reflux symptoms presentation. Azithromycin can convert IOM to normal motility in a subgroup of patients. Multiple rapid swallowing as well as swallows with abdominal compression can moderately predict the response to prokinetic therapy with Azithromycin.

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I dedicate this thesis to my wife, daughter, mum and dad.

London, 24 September 2014

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C2	<i>I still enjoy things I used to enjoy</i>	<i>Error! Bookmark not defined.</i>
C3	<i>I get a sort of frightened feeling as if something awful is about to happen</i>	<i>Error! Bookmark not defined.</i>
C4	<i>I can laugh and see the funny side of things.....</i>	<i>Error! Bookmark not defined.</i>
C5	<i>Worrying thoughts go through my mind</i>	<i>Error! Bookmark not defined.</i>
C6	<i>I feel cheerful</i>	<i>Error! Bookmark not defined.</i>
C7	<i>I can sit at ease and feel relaxed.....</i>	<i>Error! Bookmark not defined.</i>
C8	<i>I feel as if I am slowed down.....</i>	<i>Error! Bookmark not defined.</i>
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C10	<i>I have lost interest in my appearance.....</i>	<i>Error! Bookmark not defined.</i>
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C12	<i>I look forward with enjoyment to things.....</i>	<i>Error! Bookmark not defined.</i>
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List of abbreviations

AZI	Azithromycin
CFV	Contractile front velocity
DCI	Distal contractile integral
DL	Distal latency
DMN	Dorsal motor nucleus
ERD	Erosive reflux disease
GOJ	Gastro-oesophageal junction
GORD	Gastro-oesophageal reflux disease
HADS	Hospital anxiety and depression score
HFIUS	High frequency intraluminal ultra sonography
HPZ	High pressure zone
HRM	High resolution manometry
IBP	Intrabolus pressure
IOM	Ineffective oesophageal motility
LOS	Lower oesophageal sphincter
MRS	Multiple rapid swallowing
NANC	Non-noradrenergic, non-cholinergic
NERD	Non-erosive reflux disease
NO	Nitrous oxide
OH	Oesophageal hypomotility
PB	Peristaltic break
PCI	Proximal contractile integral
PFV	Proximal front velocity
PPL	Proximal peristaltic length
SPG	Swallowing pattern generator
TLOSR	Transient LOS relaxation
TZ	Transition zone

Chapter 1

Introduction

CHAPTER 1: INTRODUCTION

Ineffective oesophageal motility (IOM) is defined as a swallow response associated with poor bolus transit in the distal oesophagus on conventional line tracing. Ineffective oesophageal motility is believed to be an important pathologic feature of both gastroesophageal reflux disease (GORD) (3) and dysphagia symptoms making it an important diagnosis in classification schemes for oesophageal manometry (4). In spite of the significant prevalence and role of IOM in the pathophysiology of GORD and dysphagia the mechanisms of IOM are not clear and the treatment options have very variable and disappointing results. In the present chapter I aim to review normal oesophageal motility and current knowledge about the mechanisms of IOM and oesophageal hypomotility (OH) as well as the treatment options available.

1. NORMAL HUMAN OESOPHAGUS

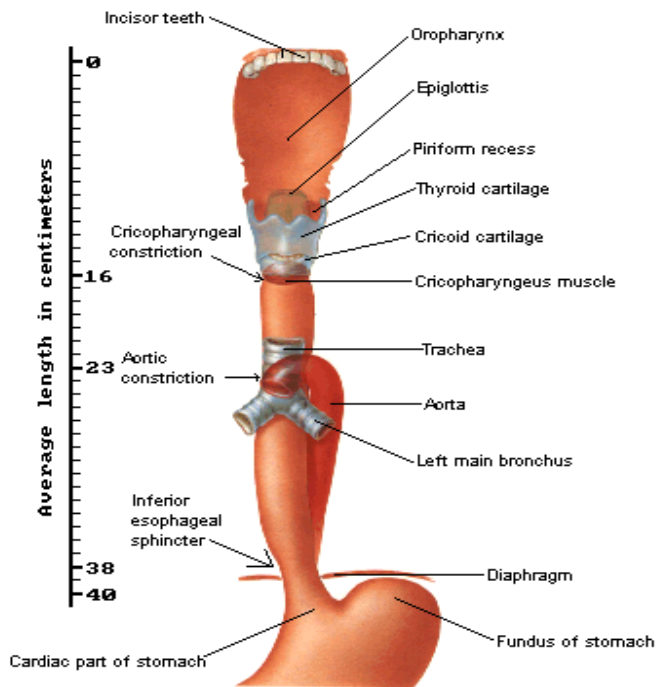


Figure 1 - Oesophageal landmarks and length (image from About Cancer.com)

of the fourth or fifth cervical vertebra, and it ends higher, at the level of the ninth thoracic vertebra (5).

Food, once chewed, tasted and lubricated in the mouth is transported into the stomach via the oesophagus. The lower oesophageal sphincter allows gastric content to remain in place and not easily regurgitated even during vigorous physical activities. This prevents corrosive digestive juices from contaminating the oesophagus itself as well as the mouth, teeth, and vocal cords. Swallowing of solids could even take place when one is upside down or in outer space, with

The oesophagus is a 25-cm long muscular tube that connects the pharynx to the stomach. The length of the oesophagus at birth varies between 8 and 10 cm and measures about 19 cm at age 15 years (5). The oesophagus extends from the lower border of the cricoid cartilage (at the level of the sixth cervical vertebra) to the cardiac orifice of the stomach at the side of the body of the 11th thoracic vertebra. The upper limit in the newborn infant is found at the level

peristalsis alone without the help of gravitational force. Gastric content can also be expelled retrogradely in the case of vomiting and reflux. The muscular composition and innervation of the oesophagus can sense a multitude of stimuli, propel food bolus inwards and outwards, and form areas of high tone (sphincters) which contract and relax appropriately. All these functions are only possible due to the extensive nerve supply, receptors, and musculature arrangements within this complex organ. Many of these physiological characteristics of the oesophagus can be altered in pathological conditions. In order to understand alteration in disease states, understanding of normal anatomy and physiology is necessary.

2. ANATOMICAL AND PHYSIOLOGICAL CONSIDERATIONS

The oesophagus can be divided into upper, middle and lower parts. This distinction is not just physical, but has a different embryological basis which determines the anatomy of the organ including; muscular composition, innervation, vascular supply and drainage. The embryological origin of the upper oesophagus is from branchial arches 4, 5 (6) and, they mainly form the striated muscular components of the upper oesophagus; the lower oesophagus by contrast originates from mesenchyme of the somites (6) which forms the smooth muscle layers of the middle and lower oesophagus.

2.1. Muscle structure of the oesophagus

The oesophagus is a muscular organ composed of two different types of muscles (6). The

muscular composition is mainly striated in the upper oesophagus, and as it progresses to the lower oesophagus, it becomes mixed with smooth muscle and by the lower third of the oesophagus, it is mainly smooth muscle. The oesophageal wall is composed of the outer longitudinal muscle and inner circular layer (7). The longitudinal muscle is arranged in fasciculi. These fasciculi are more distinct in the upper oesophagus, and merge into a single sheet towards the distal oesophagus (6). The circular muscle is arranged as concentric circles and provides the peristaltic contractions. Accessory bands of muscles connect the oesophagus to adjacent structures (Figure 1).

Musculature of Esophagus

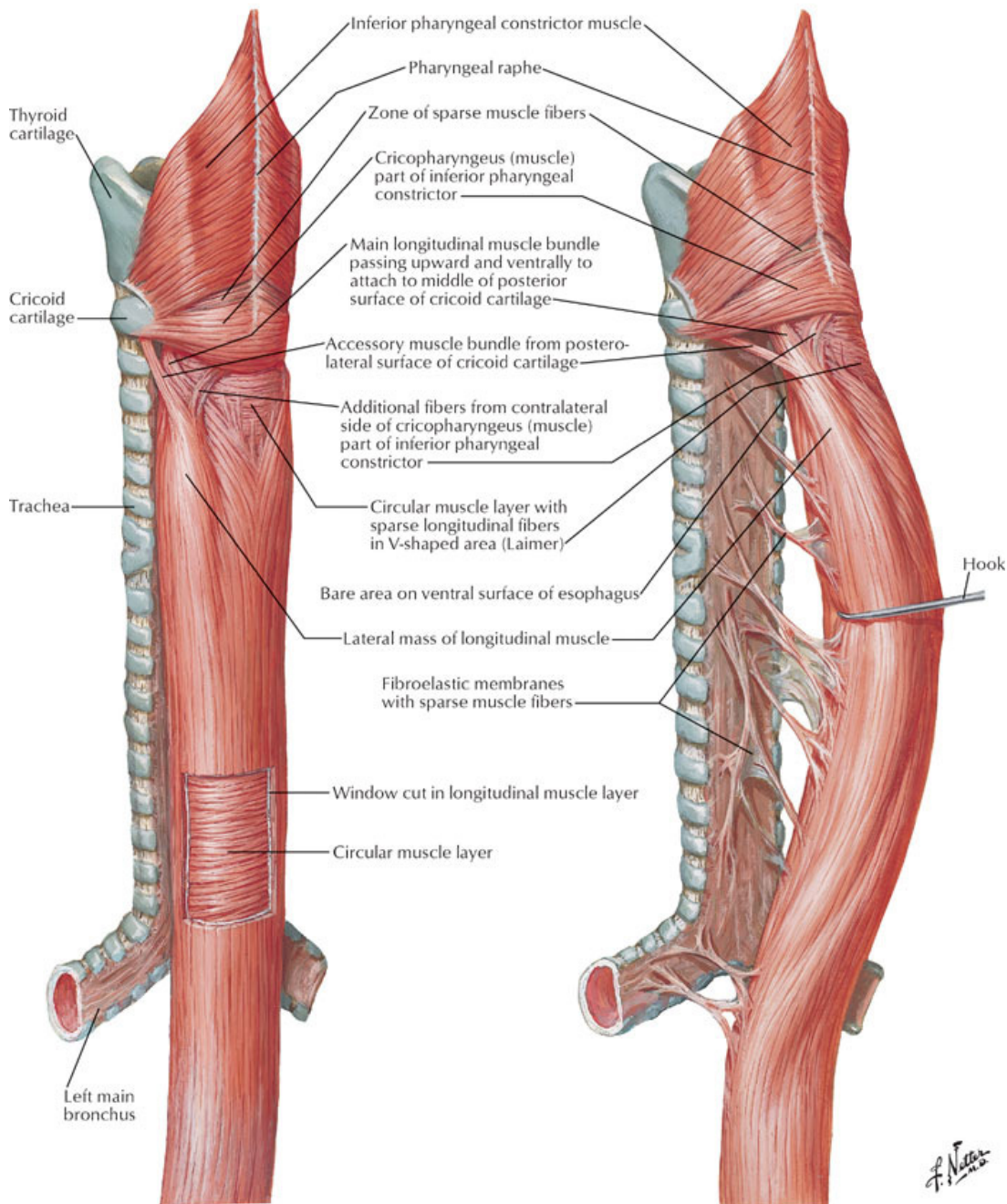


Figure 2 - Musculature of the oesophagus - From the following article: Oesophagus - anatomy and development, Braden Kuo and Daniela Urma, GI Motility online (2006). (Source of image: Netter medical illustration with permission from Elsevier. All rights reserved.)

Adaptation within the muscular tube forms the 2 oesophageal sphincters; the upper oesophageal sphincter (UOS) and lower oesophageal sphincter (LOS). Upper oesophageal sphincter separates the oropharynx from the oesophagus and the lower oesophageal sphincter separates the oesophagus from the stomach. The UOS is mainly a functional sphincter (without any specific sphincteric muscle within oesophageal structure), an area with highly sensitive nerves and reflexes triggered by swallowing. The LOS on the other hand, is both a functional as well as anatomical sphincter.

The lower oesophageal sphincter (LOS) is the incrassate muscle bundle located at the oesophagogastric junction, and includes the sling fibers from the greater curvature and clasp fibers from the lesser curvature of the stomach (8). In 1979, Liebermann-Meffert et al. characterized the clasp and sling muscle fibers in cadavers. They described the sling muscle fibers on the greater curvature of the stomach and the clasp muscle fibers on the lesser curvature, both found within the gastric cardia (9). The clasp and sling muscle fibers have been characterized as having an asymmetric distribution and being the major anatomic component within the HPZ (Figure 2). Three distinct anatomic structures, the clasp and sling muscle fibers, crural diaphragm, and lower oesophageal circular muscle combine to form the antireflux barrier of the proximal stomach and distal oesophagus. The clasp and sling muscle fibers combine with the crural diaphragm to form a distal pressure profile.

The LOS tone is maintained by a constant smooth muscle contraction controlled by the nervous plexi and neuro-hormonal factors. In addition, the anatomy of LOS represents an area of

thickened musculature, corresponding to the diaphragmatic ring, and enters the abdomen at an angle. These mechanisms form the LOS and contribute to the prevention of reflux of gastric contents as well as allowing the entry of food bolus into the stomach when necessary.

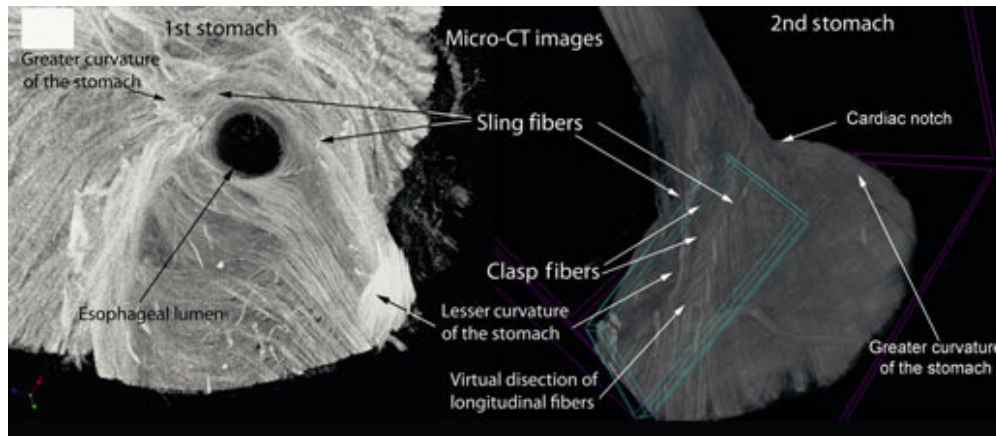


Figure 3 - Micro-computed tomography (Micro-CT) images showing clasp and sling muscle fibers from two different stomach and oesophagus specimens procured from organ donors. The image on the left is a view from the inside of the stomach after virtually dissecting the mucosa. The sling fibers can be observed encircling more than 75% of the oesophageal lumen and then running along the lesser curvature. The image on the right is a view from the outside of the stomach and oesophagus after virtually dissecting the longitudinal muscle fibers along the lesser curvature. The sling fibers can be seen running longitudinally and being bridged by the clasp fibers (10).

1.1 Layers of the oesophageal wall

Oesophageal wall is organised into distinct layers (Figure 3 and 4). This layout allows movements between the layers that are optimum for a dynamic tube. Within the layers, blood vessels, nerves complexes, receptors and connective tissues exist. Histologically, the oesophagus has the following 4 concentric layers (11):

- Mucosal layer
- Submucosal layer

- Muscular layer
- Adventitial layer

A) The mucosal layer consists of three sub-layers (12):

1-The luminal surface of the oesophagus is lined by "mucous" or non-keratinized stratified squamous epithelium(7). The squamous epithelium is adapted to withstand abrasions. The epithelium is rich in receptors and sensitive nerves endings(13) that respond to a range of stimuli and therefore, intact epithelium is important in normal oesophageal sensation.

2-Below the epithelium are the lamina propria and

3-muscularis mucosae (12).

B) The submucosa is mainly connective tissue and it loosely connects the mucous membrane and the muscular coat. This layer contains the larger blood vessels, the submucosal (Meissner) nerve plexus, and oesophageal glands.

C) The third layer is the muscular coat, consisting of inner circular and outer longitudinal muscles (6). The longitudinal layer is generally thicker than the circular layer:

- Inner circular muscle fibers - These fibers are continuous superiorly with the fibers of the cricopharyngeal part of the inferior constrictor and inferiorly with oblique fibers of the stomach (5).
- Outer longitudinal muscle fibers - The longitudinal muscle fibers form a continuous coat around the whole of the oesophagus except posterosuperiorly, 3-

4 cm below the cricoid cartilage; here, they diverge as 2 fascicles that ascend obliquely to the anterior aspect of the oesophagus (5).

D) The fourth and the outermost fibrous layer is formed by external adventitia of irregular, dense connective tissue containing many elastic fibers. The thoracic oesophagus has no serosa, which makes it unique to the rest of the gastrointestinal tract (14).

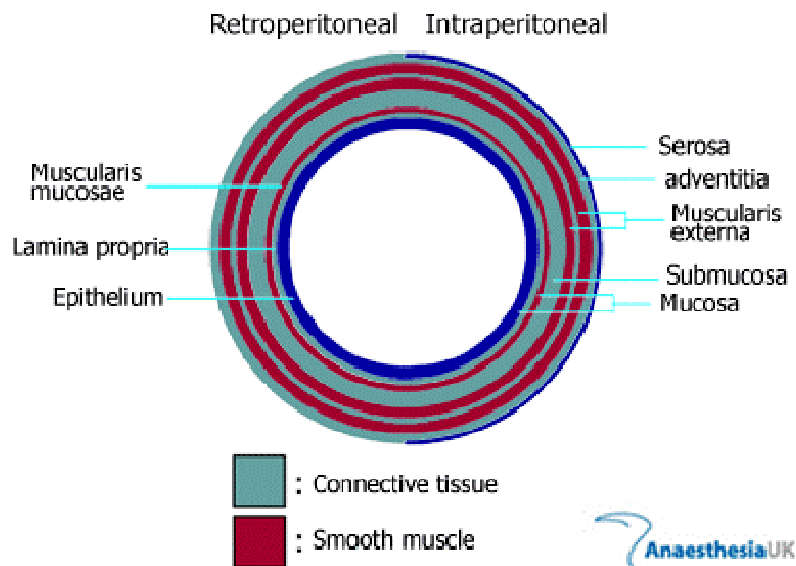


Figure 4 - Different layers of the oesophagus including retroperitoneal and intraperitoneal aspects of the oesophagus. (Anaesthesia UK: training site of Royal College of Anaesthetics, UK)

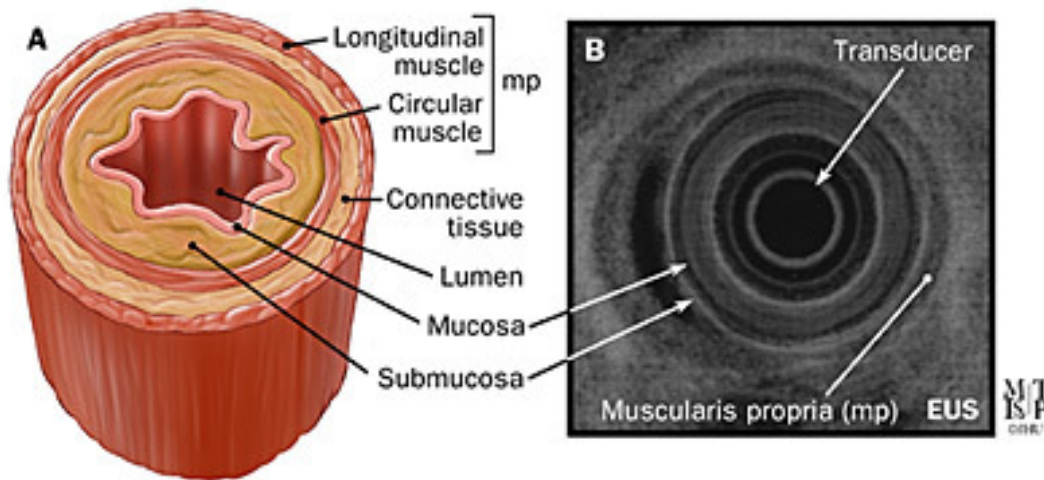


Figure 5 - A, Normal anatomy of the oesophageal wall; B, endoscopic ultrasonography (EUS) image (Source of the image Johns Hopkins Medicine).

1.2 Innervation

1.2.1 *Intrinsic nerve supply*

There are 2 separate plexi within the wall of the oesophagus (Figure 5).

3. Associated within the submucosal layer is Meissner's plexus which innervates the muscularis mucosae and secretory glands.
4. Within the deeper muscular layer, between the longitudinal and circular muscle layers is the myenteric Auerbach's plexus. Myenteric plexus controls the contractions of the circular and longitudinal layers. The Myenteric plexus also exist in the striated muscle of the upper oesophagus, although its function there is less clear.
5. A network of fibres is believed to connect the two plexi (6).

Together the submucous (Meissner's) and myenteric (Auerbach's) plexi form the intrinsic innervation of the oesophagus. The coordination of spontaneous peristalsis by the smooth muscle is controlled by the intrinsic system which is independent from extrinsic control.

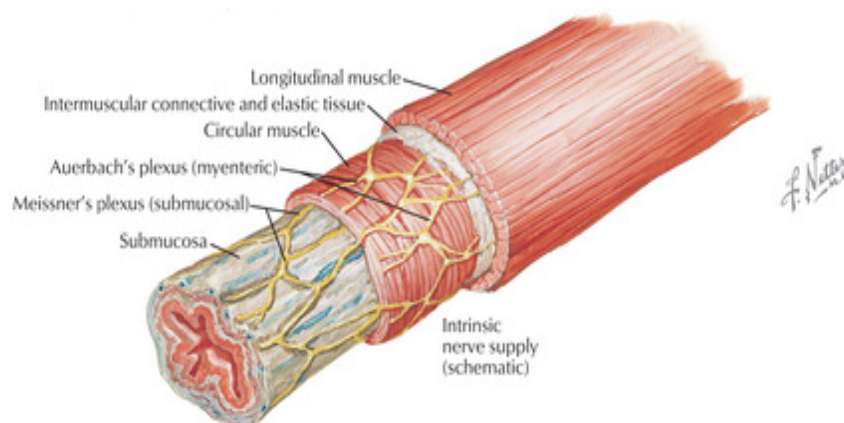


Figure 6 – Intrinsic innervation of oesophagus. (Source of image: Netter medical illustration with permission from Elsevier. All rights reserved.)

1.2.2 ***Extrinsic nerve supply***

A) Motor innervation

The main motor supply of the oesophagus is the vagus nerve which supplies the upper and lower oesophagus.

- 1-Branches supplying the upper oesophageal muscles and upper oesophageal sphincter come from the nucleus ambiguus.
- 2-The vagal efferents for the distal oesophagus and lower oesophageal sphincter originate from the dorsal motor neuron of the vagus nerve(6).

Although the vagus controls most of the motor function of the oesophagus, it is mainly a *sensory* nerve with up to 85% of vagus nerve fibres being sensory(15). This is discussed further below.

B) Sensory innervation

The sensory nerves of the oesophagus are less well studied to date compared to motor nerves. Sensation in the viscera is also much less discrete compared to somatic sensation. However, sensory modalities that exist within the oesophagus are quite wide ranging including; thermo, chemo, and mechano-sensations. The sensory innervation can be divided into 2 systems:

1-vagal afferents are mainly parasympathetic and

2-spinal afferents which are predominantly sympathetic (16).

These 2 systems share some similar activation pathways(17) and interaction between them occurs(16). For example, the vagus system, is shown to reduce hyperalgesia by reducing release of epinephrine from adrenals(18) as well as reducing sympathetically controlled neurogenic inflammation(19). (Figure 6)

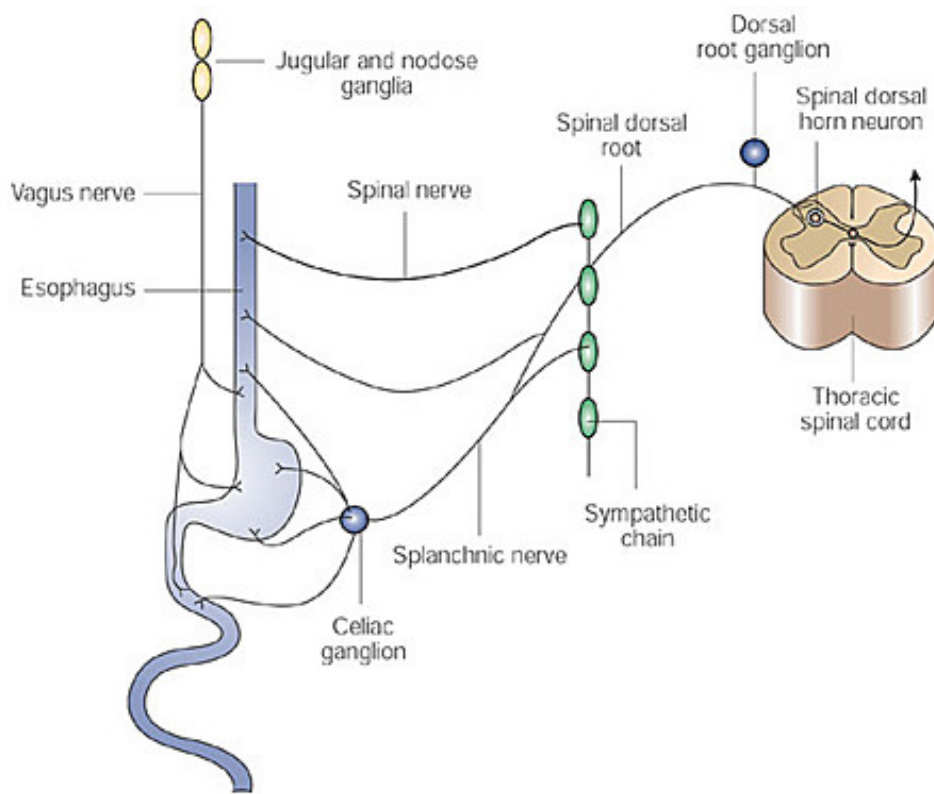


Figure 7 - Schematic diagram of vagal and spinal nerve supply to the oesophagus (source of the image is GI Motility online (2006)(1).

1.2.3 *Vagus afferents*

The vagal fibres are mainly un-myelinated C-fibres and have their cell bodies in the nodose ganglia before projecting into the nucleus solitary tract (NST). From the NST, these fibres form synapses with second order neurons and project to the brainstem, hypothalamus, amygdale and cerebral cortex(15, 20). Vagal afferents are traditionally thought to mediate physiological sensations such as satiety and nausea(21). However, related to its physiological motor function, the vagus nerve is also believed to be sensitive to mechanosensation (21). Vagal afferents have receptors in the mucosa which respond to mucosal fine stroking as well as tension receptors in the oesophageal wall responsive to distension(22). Vagal afferents are also enriched with

receptors responsive to polymodal intra-luminal stimuli(18) including; osmo(23)-, chemo(24)- and thermo(25)-sensations.

1.2.4 *Spinal afferents*

The current understanding is that there are mucosal nerve endings that sense intra-luminal stimuli(13). They are located in the lamina propria of the mucosa. These nerve endings are mainly spinal afferents and have cell bodies in dorsal root ganglia. From the spinal cord, they travel into the thalamus and primary sensory cortical areas. The distribution of nerve endings is thought to vary according to stimuli. Acid sensing nerve endings are believed to be superficial in the epithelium whereas nerve endings deeper in the muscle and serosa are believed to be important for mechanosensation..

1.2.5 *Efferent nerves: Autonomic Nervous System*

1-As discussed above in motor supply, the vagus which is predominantly parasympathetic(23) provides motor innervation to the muscular layers as well as secretory function to the mucosal glands. The origins of the vagus nerve are the nucleus ambiguus and dorsal motor nucleus.

2-Sympathetic innervation of the oesophagus originates mainly from the thoracic sympathetic chain (T1-10) with the first thoracic ganglion frequently joint to the cervical ganglion to form the stellate ganglion(7). The sympathetic system regulates vascular smooth muscle tone, and to a lesser extent than the parasympathetic system, oesophageal contractile and secretory functions. Sympathetic activation had been traditionally

believed to increase LOS tone and causes contraction(26) via adrenergic system(27). However, a study performed in cats did not show sympathetic modulation of LOS function(28).

1.2.6 *Efferent nerves: Central nervous system*

Oesophageal body - Within the brain the two main areas for oesophageal sensation, including pain processing, are the thalamus and cerebral cortex. The thalamus located in the diencephalon, at the dorsal end of the brainstem forms the central core of the brain. It is an important centre for relaying and integrating important sensory and motor messages from the periphery to cortical areas. It also integrates factors such as consciousness, attention, memory and emotions. The cortical areas for oesophageal sensation are; cingulate, insular, sensory, parietal occipital, and prefrontal regions based on human studies(29-31).

Since the motor function of the oesophagus is mainly involuntary, much of the known information of the role that the central nervous system plays in its function pertains to sensation.

Swallowing - There is limited evidence of motor representation of oesophagus in the cortex in swallowing studies. A sophisticated human study using transcranial magnetic stimulation and magnetic resonance imaging by Hamdy et al showed that swallowing musculature is discretely and somato-topically represented in the motor and pre-motor cortex of both hemispheres asymmetrically and not influenced by the dominant handedness. The loci for mylohyoid, pharynx and oesophagus were discreet with the oesophageal locus predominantly in the pre-

motor cortex (32). Cortical swallowing motor pathways from each hemisphere interact and their excitability is modulated by sensory input(33). For example, stimulation of afferent branches of cranial nerves Trigeminal and Vagus had been shown to facilitate cortical swallowing pathways in the brainstem(34) which will be further discussed below.

Role of the nervous system in regulation of oesophageal motility

The sequence of peristaltic events has limited contribution of extrinsic autonomic innervation but rather involves the activation of intrinsic sensory neurons, which are coupled via modulatory interneurons to excitatory and inhibitory motor neurons projecting into the smooth muscle layer (35).

In contrast to other parts of the gastrointestinal tracts, the external muscle layer of the mammalian oesophagus contains striated muscle fibers, which extend from the pharyngoesophageal junction to the thoracic or even abdominal portion, depending on the species (36, 37).

In humans, horses, cats and pigs, the upper and lower portions of the oesophagus are composed of striated and smooth muscles, respectively, with a mixed portion between them. On the other hand, the tunica muscularis of the LOS consists of smooth muscles (37). Proximal oesophageal motility is controlled centrally by an extrinsic neuronal mechanism whereas mid and distal oesophageal motility is controlled peripherally by an intrinsic neuronal mechanism (37, 38).

2.1 Neural mechanisms of the Oesophageal body

The mechanisms of peristalsis control are different between striated muscle and smooth muscle in the oesophageal body. However, in both portions, oesophageal peristalsis is influenced by a swallowing pattern generator (SPG) located in the brainstem (39)

2.1.1 Neural control of peristalsis in the oesophageal striated muscle portion

According to the conventional view, the SPG both initiates and organizes peristalsis in the striated oesophageal muscle, i.e., both primary and secondary peristaltic contractions are centrally mediated in the striated muscle portion(38, 39). Striated muscle fibers are innervated exclusively by excitatory vagal efferents that arise from motor neurons localized in the nucleus ambiguus and terminate on motor endplates(40). It is possible to confirm this view additionally by demonstrating that vagal nerve stimulation evokes twitch contractile responses of the striated muscle in an isolated segment of mammalian oesophagus , which are abolished by d-tubocurarine, an antagonist of nicotinic acetylcholine receptors on the striated muscle, but not by atropine, an antagonist of muscarinic acetylcholine receptors on the smooth muscle, or hexamethonium, a blocker of ganglionic acetylcholine receptors (41). Peristalsis in the striated oesophageal muscle is executed according to a sequence pre-programmed in the compact formation of the nucleus ambiguus. The compact formation of the nucleus ambiguus receives projections from the central subnucleus of the nucleus of the solitary tract (42), which in turn receives vagal afferents from the oesophagus , thus closing a reflex loop for oesophageal motor control (42).

2.1.2 Neural control of peristalsis in the oesophageal smooth muscle portion

Motor innervation of the smooth muscle oesophagus is more complex. Here, the SPG initiates peristalsis via preganglionic neurons in the dorsal motor nucleus of the vagus that project to the myenteric ganglia in the oesophagus, i.e., the primary peristalsis involves both central and peripheral mechanisms (43). The smooth muscle is innervated by myenteric motor neurons that can release acetylcholine, tachykinins or nitric oxide (NO) (43). However, the progressing front of contraction is organized by virtue of their local reflex circuits that are composed of sensory neurons, interneurons and motor neurons as elsewhere in the gut, i.e., the secondary peristalsis is entirely due to peripheral mechanisms in the smooth muscle oesophagus (38, 43). In fact, the smooth muscle oesophagus can exhibit propulsive peristaltic contractions in response to an intraluminal bolus of food even in a vagotomised model. Moreover, peristaltic reflexes can be elicited by distension in an isolated segment of the smooth muscle oesophagus from the opossum.

2.2 Involvement of intrinsic neurons in motility of the oesophageal striated muscle

The striated muscle fibers in the oesophagus were hitherto considered as 'classical' skeletal muscle fibers, innervated exclusively by excitatory vagal motor neurons, which terminate on motor endplates (40). It is believed that peristalsis in the striated muscle is executed according to a sequence pre-programmed in a medullary swallowing network and modulated via vago-vagal reflexes as described above (43). On the other hand, the presence of a distinct ganglionated myenteric plexus in the striated muscle portion of the mammalian oesophagus, comparable to

other gastrointestinal tracts, has been well known for a long time. However, functional roles of the intrinsic nervous system in peristalsis of the striated muscle in the oesophagus have remained enigmatic and have been neglected in concepts of peristaltic control (37, 43).

Investigation of the regulatory role of intrinsic neurons in the oesophagus was advanced by the discovery of 'enteric co-innervation' of oesophageal motor endplates. The enteric co-innervation challenged the conventional view of peristalsis control in the striated oesophageal muscle. Originally described in the rat, oesophageal striated muscle receives dual innervation from both vagal motor fibers originating in the brainstem and varicose intrinsic nerve fibers originating in the myenteric plexus. This new paradigm of striated muscle innervation has been confirmed in a variety of species including humans, underlining its significance (37, 44). It has been demonstrated that neuronal nitric oxide synthase (nNOS) was highly colocalized with vasoactive intestinal peptide (VIP), neuropeptide Y (NPY), galanin and Met-enkephalin in enteric nerve terminals on oesophageal motor endplates. These markers are suggestive of inhibitory modulation of vagally-induced striated muscle contraction (37). Since morphological studies revealed further that spinal afferent nerve fibers closely innervate myenteric neurons in the oesophagus (37), the presence of 'a peripheral mechanism' regulating the motility of oesophageal striated muscle including afferent and enteric neurons in the oesophagus was suggested (37).

2.3 Factors affecting strength of peristaltic contractions

Several factors influence the amplitude, duration, and propagation velocity of the contraction wave in the oesophagus: oesophageal site; posture of the patient; consistency, size, and temperature of the food bolus; and resistance to the movement of the bolus.

The contraction amplitude is highest in the lower oesophagus [69.5 \pm 12.1 mmHg, mean \pm standard error (SE)] and lowest in the mid-oesophagus (35.0 \pm 6.4 mmHg). The area of lower pressure wave corresponds to the region of mixed striated and smooth muscles. The mean contraction in the proximal oesophagus measures 53.4 \pm 9.0 mmHg. The duration of the contraction waves increases progressively in the distal parts of the oesophagus. The propagation of the wave is fastest in the upper oesophagus, and decreases in the middle and lower oesophagus.

The strength of contraction is less when the patient is upright compared to supine, and a liquid food bolus is associated with longer duration, stronger contraction, and slower propagation compared to a dry bolus of swallowed air. A larger bolus of food leads to stronger contractions. Warm boluses of food increase, whereas cold boluses decrease, the strength of contraction. The osmolality does not appear to affect the contraction wave. Increased abdominal pressure, as in the Valsalva maneuver, or strictures leading to outflow obstruction in the oesophagus will slow the propagation of the contraction.

2.4 Factors affecting latency of peristaltic contractions

Deglutitive Inhibition - The swallow-evoked peristaltic contraction consists of a wave of inhibition followed by that of contraction. The wave of inhibition that precedes peristaltic contraction is called deglutitive inhibition. The phenomenon of deglutitive inhibition is essential for drinking of fluids at a rate faster than one swallow every 10 seconds. During the usual drinking of water, swallows may be accomplished every 1 to 2 seconds. This is made possible by the phenomenon of deglutitive inhibition in which a swallow abruptly inhibits any on-going contraction in the oesophagus. When multiple swallows are taken in rapid succession, the oesophageal body remains inhibited until the last of the series of swallow, after which there is a fully conducted peristaltic contraction wave.

Role of latency in generating peristaltic contraction (45) - The peristalsis in the smooth muscle is based on the fact that the duration of the deglutitive inhibition associated with swallowing increases distally along the length of the oesophagus. This gradient of inhibition is manifested only as the gradient of increasing latency of contraction in the non-contracted oesophageal body smooth muscle. This gradient is due to both central and peripheral mechanisms. The central mechanism involves near-instantaneous activation of the inhibitory short-latency vagal fibers, which arise from neurons located in the caudal part of DMN. This is transmitted to all levels of the oesophagus by the SPG so that the oesophagus in its entire length is inhibited promptly on swallowing. The distally increasing inhibitory nerve influence is

responsible for the distally increasing duration of inhibition along the oesophagus. The myenteric inhibitory neurons were thought to act by releasing a Non-noradrenergic, non-cholinergic (NANC) inhibitory neurotransmitter that is now shown to be nitric oxide. In addition, regional properties of the oesophageal smooth muscle may also contribute to the distally increasing gradient of the duration of the deglutitive inhibition.

Primary peristalsis in the thoracic oesophagus is also orchestrated by the swallowing program generator (SPG) in the brainstem. However, the mechanism of peristalsis in the smooth muscle segment is complex and involves coordinated activities of the vagal inhibitory and excitatory pathway, regional gradients of the myenteric inhibitory and cholinergic excitatory nerves, and the regional characteristics of the oesophageal smooth muscle.

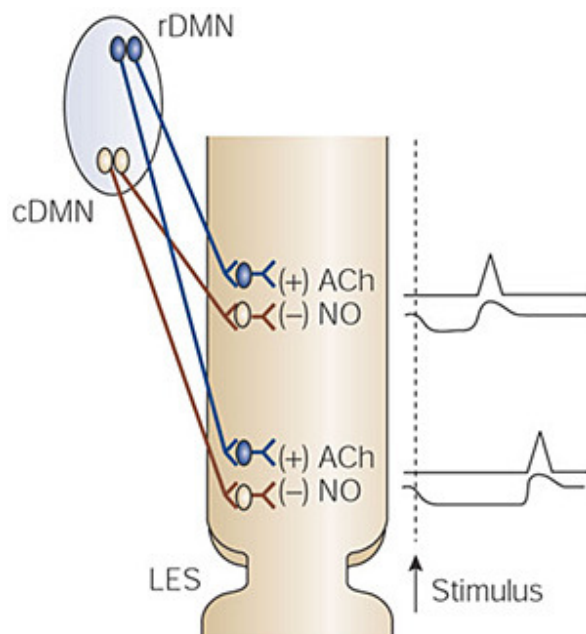


Figure 8 - Central control of peristalsis in the smooth muscle portion of the oesophagus. - Upon swallowing (stimulus), the inhibitory pathway neurons in the caudal DMN (cDMN) are activated first, which causes simultaneous inhibition of all parts of the oesophagus. This inhibition lasts longer in the lower than in the upper parts. As the inhibition ends, sequential activation of excitatory (including cholinergic) neurons in the rostral DMN (rDMN) elicits a contraction wave that is peristaltic in nature (45).

The deglutitive inhibition is immediately followed by deglutitive excitation that is manifested by the oesophageal contraction. Deglutitive excitation involves noncholinergic rebound excitation as well as cholinergic excitation.

Experimental studies have shown that stimulation of the NANC inhibitory nerves causes inhibition of the oesophageal smooth muscle that is followed by a rebound contraction. The mechanism of rebound contraction is not known. It is clearly not cholinergically mediated. It is not clear whether the inhibitory transmitter itself somehow causes rebound contraction or an unknown NANC excitatory neurotransmitter is released after the release of the inhibitory neurotransmitter. The contribution of the noncholinergic rebound contraction to the force of oesophageal peristaltic contraction increases distally along the length of the oesophagus.

The deglutitive cholinergic excitation also follows the deglutitive inhibition and overlaps the rebound contraction. The cholinergic excitation involves activation of the excitatory vagal (long latency fibers) pathway consisting of preganglionic neurons in the rostral part of the DMN and postganglionic cholinergic neurons in the myenteric plexus. The sequential activation of the excitatory vagal pathway supplying the oesophagus in a craniocaudal orientation leads to a sequential wave of excitation that is timed to cause a peristaltic contraction in the oesophagus. There is a distally decreasing gradient of cholinergic innervation along the oesophagus. As a consequence, cholinergic excitation provides a greater contribution to the force of peristaltic contraction in the upper than the lower parts of the smooth muscle oesophagus.

The swallow-evoked sequential cholinergic excitation is timed to occur when the deglutitive inhibition at different oesophageal levels is terminated. However, there is overlap between the deglutitive inhibition and the deglutitive excitation. This overlap is most prominent in the proximal and least prominent in the distal parts of the oesophagus. As a consequence, cholinergic deglutitive excitation causes greater shortening of latency of swallow-associated contraction in the proximal than in the distal parts of the oesophagus. (Figure 8).

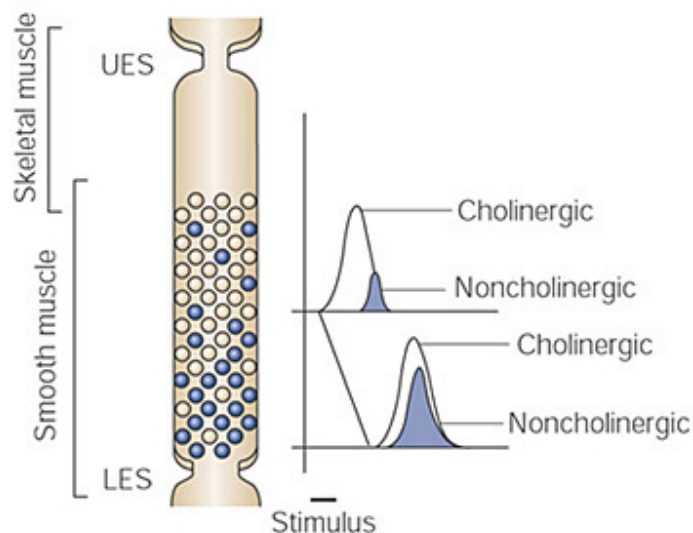


Figure 9 - Gradient of cholinergic excitatory and noncholinergic inhibitory nerves in the smooth muscle portion of the oesophagus (45). The cholinergic excitatory innervation (open circles) is most marked in the proximal part and decreases gradually in the distal part. On the other hand, the inhibitory innervation (close circles) increases distally along the oesophagus. As a result, upon stimulation the latency of contraction increases gradually distally along the oesophagus, resulting in peristaltic sequence of contraction that is entirely located locally in wall of oesophagus. (Source: Crist J, Gidda JS, Goyal RK. Intramural mechanism of oesophageal peristalsis: roles of cholinergic and noncholinergic nerves. Proc Natl Acad Sci USA 1984; 81(11):3595–3599 with permission).

In conclusion, peristalsis in the oesophageal smooth muscle is due to distally increasing duration of deglutitive inhibition followed by deglutitive excitation. The pattern of activation of the inhibitory and excitatory vagal pathways, the regional gradients of inhibitory and excitatory

myenteric nerves, and the intrinsic properties of the smooth muscle all determine velocity of peristalsis. The oesophageal peristaltic contractions themselves are a blend of noncholinergic and cholinergic components. As a consequence, cholinergic antagonists such as atropine increase the latency and decrease the amplitude of contraction in the proximal but not the distal parts of the oesophagus. In contrast, antagonists of NOS reduce the latency mainly in the distal segments of the oesophagus and lead to simultaneous contractions.

Nitric oxide (NO) produced from nNOS in the nerve terminals is the major inhibitory neurotransmitter in the oesophagus. Nitric oxide causes inhibition by a cyclic guanosine monophosphate (cGMP)-dependent mechanism. Vasoactive intestinal peptide serves as an intermediate in enhancing electrical spike-induced augmentation of calcium influx and NO synthesis from nNOS. Adenosine triphosphate (ATP) is not involved as an inhibitory neurotransmitter in the oesophagus.

2.5 LOS

The LOS is a specialized region of the oesophageal circular smooth muscle that allows the passage of a swallowed bolus to the stomach and prevents reflux of gastric contents into the oesophagus (38, 43, 46). Appropriate opening and closure of the LOS is controlled by neuronal mechanisms that normally maintain tonic contraction of the musculature to prevent reflux and cause relaxation during swallowing. The LOS is innervated by both excitatory and inhibitory motor neurons that are located in the myenteric plexus of the LOS and the oesophageal body.

Acetylcholine and NO are the main excitatory and inhibitory neurotransmitters involved in LOS contraction and relaxation, respectively (46). In addition, VIP, ATP, carbon monoxide (CO), and calcitonin gene-related peptide (CGRP) also have been proposed as putative neurotransmitters in the LOS (46). A subclass of intrinsic neurons is innervated by vagal preganglionic fibers as postganglionic neurons (38).

Hormonal Influences on the Oesophagus - Swallowing is not under direct hormonal control but oesophageal motility, particularly LOS, may be influenced by a number of hormonal factors. This has significance with regard to gastroesophageal reflux. Gastrin tends to increase LOS tone. Motilin also increases LOS tone, which could explain the rise in LOS pressure during the migrating motor complex. Secretin, CCK, and GIP all tend to inhibit the LOS tone. VIP, like secretin, inhibits LOS tone. Progesterone also inhibits LOS tone and may account for an increased incidence of gastroesophageal reflux during pregnancy. Any factor that inhibits LOS tone may enhance the occurrence of gastroesophageal reflux and the adverse effects of acidic stomach contents in the oesophagus.

Transient lower oesophageal sphincter relaxations (TLOSRS) - LOS contracts and relaxes appropriately to allow the inward passage of food into the stomach for digestion and prevents the reflux of gastric content. Even in the presence of normal anatomy, inappropriate and uncoordinated relaxation of the LOS can cause reflux. This phenomenon is called transient lower oesophageal sphincter relaxations (TLOSRS). In a study in human volunteers, gastro-oesophageal reflux was shown to be not related to low steady-state basal LOS pressure, but

rather occurred during inappropriate transient relaxations(47). TLOSRS mostly tend to occur after eating due to distension of the proximal stomach(48, 49) although they can occur spontaneously too. It is thought to be a vagally mediated reflex from gastric mechano-distension(48, 50). A study in humans after fundoplication showed that TLOSRS were reduced, although gastric accommodation was not altered indicating that the receptive field for TLOSRS was located within a wider sensory field, perhaps in a region affected by fundoplication; proximal stomach (49) close to the oesophagus.

1.3 Oesophageal role on prevention of reflux

Related to the main function of swallowing and entry of food into the stomach, the lower oesophageal sphincter (LOS) also functions to prevent reflux of gastric contents into the oesophagus. The LOS as described above, is a muscular sphincter as well as functional sphincter dependent on maintenance of muscular tone and its angle. Inability of LOS to relax and contract appropriately would result in diseased states. Dysphagia (eg. in achalasia) occur when oesophagus and LOS fails to relax in a coordinated way to allow entry of food. Gastro-oesophageal reflux disease (GORD) is associated with spillage of gastric contents into the oesophagus. Many factors normally contribute to the prevention of this backflow from happening and in GORD, one or more of these mechanisms may fail (discussed below in: Pathogenesis of GORD).

One old concept recently being in the centre of attention in the pathophysiology of GORD is the “acid pocket”. Acid reflux and its associated symptoms occur most frequently following the

ingestion of a meal. This observation presented a dilemma as intragastric pH is at its least acidic following eating due to the buffering effect of the food. However, the observation by Fletcher et al that the proximal cardia region of the stomach escapes the buffering effect of the meal provided a rational explanation for the acidic nature of the postprandial refluxate (51, 52). The zone of high acidity detected in the proximal stomach after a meal has been termed as the acid pocket. The relation of acid pocket with effect of prokinetic therapy is under debate but in the section 7.1.3.2 is discussed in more details.

2 OESOPHAGEAL HYPOMOTILITY

The oesophagus functions solely to deliver food from the mouth to the stomach where the process of digestion can begin. Efficient transport by the oesophagus requires a coordinated, sequential motility pattern that propels food from above and clears acid and bile reflux from below. Disordered pharyngeal and oesophageal motor function is a common cause of symptoms, particularly dysphagia, chest pain, and gastroesophageal reflux symptoms (heartburn and regurgitation). Motor function can be assessed by a variety of recording techniques including radiology, scintigraphy manometry, and most recently intraluminal electrical impedance monitoring. Some of these are complementary. The gold standard, however, for the assessment of motor disorders remains manometry. Manometric measurement of oesophageal pressure is the most direct method for assessment of motor function. Only manometry can give information on the strength of contractions. Hypo-contraction abnormalities of oesophagus that result from weak (low amplitude) muscle contractions can cause ineffective oesophageal motility (IOM) that delays oesophageal clearance, and LOS hypotension. In this section the terms of OH and ineffective oesophageal motility will be discussed in more details.

3 Definitions (definition of IOM used in this thesis)

OH is a term used to define low amplitude contractions in the body of the oesophagus (oesophageal hypotensive peristalsis) and hypotensive LOS.

Until 1997, the term ‘nonspecific oesophageal motor abnormalities’ was generally used by physiologists to denote any dysmotility pattern that was not achalasia, spasm, nutcracker or LOS dysfunction. Then, Leite et. al published their finding that ‘ineffective oesophageal motility’ (IEM or IOM) was the primary finding in patients with nonspecific oesophageal motility disorder(53, 54).

In 2001, this was incorporated into Spechler and Castell’s(55) classification of oesophageal motor disorders, based on conventional manometry. In their classification, OH was defined as distal-oesophageal hypocontractility in at least 30% of wet swallows, characterized either as low-amplitude peristaltic waves (<30 mmHg), low-amplitude simultaneous waves (<30 mmHg), peristaltic waves that are not propagated to the distal-oesophagus, or absent peristalsis. The 30-mmHg criterion was derived from the observation that amplitudes <30 mmHg were frequently associated with bolus escape and incomplete bolus clearance(56) measured either radiologically(56) or scintigraphically(58). More recently, High-resolution manometry (HRM), with or without concurrent intraluminal impedance monitoring, allows a more complete definition of peristalsis (Figure 9).

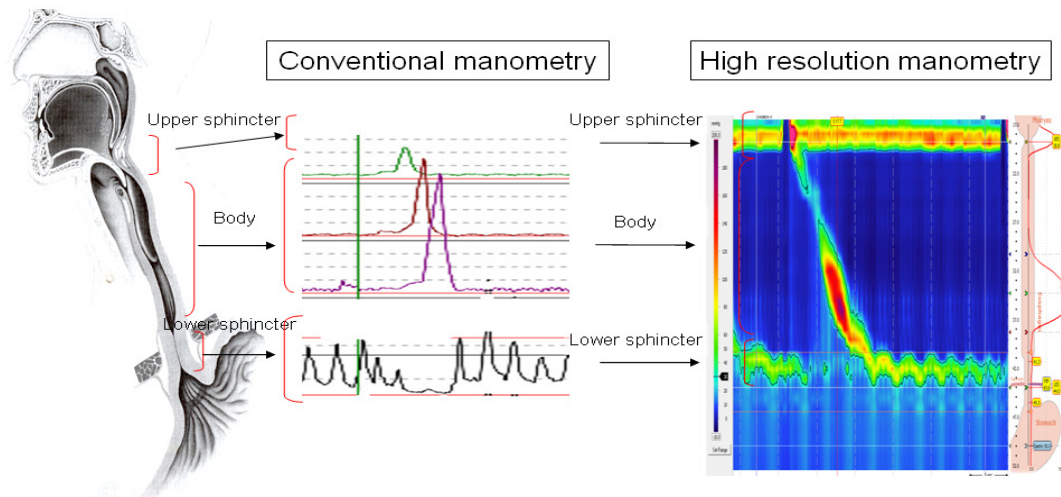


Figure 10 - Comparison of conventional versus high resolution manometry. (Image from internal source)

In the recently developed Chicago classification(59) frequent failed peristalsis (>30% of wet swallows) is separated from weak peristalsis (defined as breaks in the 20-mmHg isobaric contour). Weak peristalsis with large defects is judged to be present when breaks >5 cm are present in >20% of swallows (Fig. 2). Weak peristalsis with small defects is present when breaks of 2–5 cm in length are present in >30% of swallows(60). This classification of manometric abnormalities is also based on the likelihood that such defects are associated with oesophageal dysfunction (i.e. bolus escape). However, the clinical relevance of such observations remains uncertain. Indeed, it is likely that several abnormal swallows in a series are required before symptoms are experienced(61) (Figure 10). In the latest study to define IOM using high resolution manometry, Xiao et al ²³⁸ suggested that IOM should be identified in two ways: 1) 50% or more swallows with any combination of failed peristalsis, weak contraction with small break or weak peristalsis with large break in the middle/distal oesophagus or 2) 50% or more swallows associated with a DCI < 450 mmHg-s-cm (DCI or distal contractile integral will be

described in the following sections in more detail). In 2014, Chicago group defined IOM as: $\geq 50\%$ ineffective swallows (Ineffective swallows can be failed or weak with $DCI < 450$ mmHg•s•cm) and generated a new class of diagnosis as “Fragmented peristalsis” ($\geq 50\%$ fragmented contractions with peristaltic gap > 5 cm and $DCI > 450$ mmHg•s•cm). Therefore, weak peristalsis with small breaks disappeared from the Chicago classification (62).

In this thesis, I adopted Xiao’s definition of the IOM however weak peristalsis with small breaks are excluded as Chicago group suggested and in I believe this minute motility finding will not be affecting clinical circumstances of the patients. Hence, the definition of IOM in this thesis is: 1) having fragmented peristalsis [50% or more swallows with any combination of failed peristalsis and weak peristalsis with large break (> 5 cm) in the middle/distal oesophagus], or 2) 50% or more swallows associated with a $DCI < 450$ mmHg-s-cm.

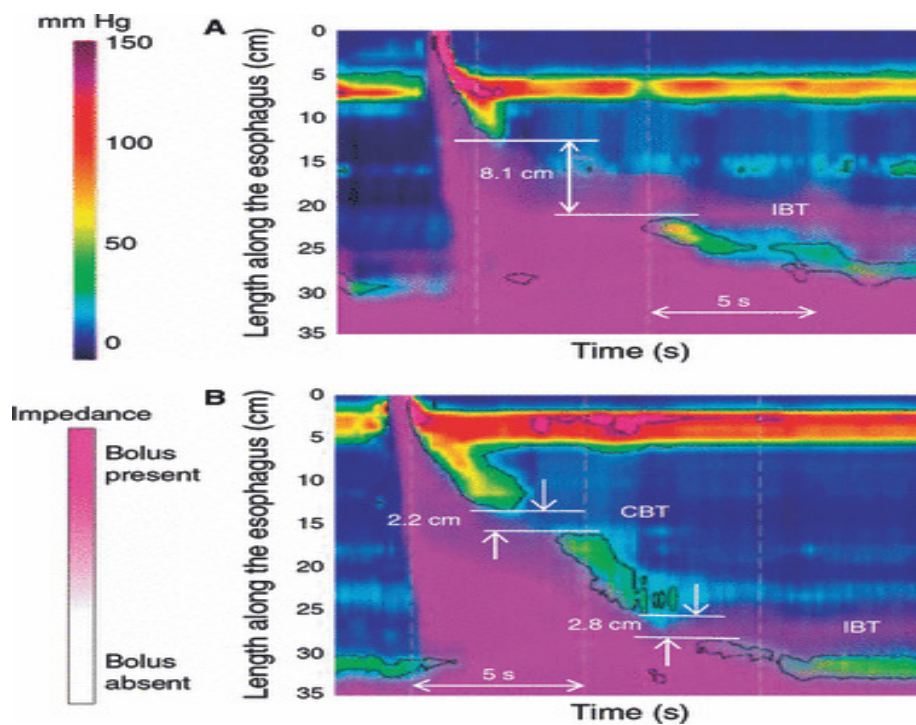


Figure 11 - Examples of high-resolution manometry showing weak peristalsis with small (2–5 cm) (A) and large (>5 cm) (B) breaks in the 20-mmHg isobaric contour. Reproduced with permission from Roman et al. *Am J Gastroenterol* 2011;106:349–356.

4 Prevalence of OH

Normal values for conventional manometry are based on observations in 95 healthy subjects with a mean age of 43 years (range 22–79 years)(53), whereas the two HRM studies upon which the cutoff values for peristaltic breaks were based on volunteers under the age of 50(60, 64). Data gathered with conventional manometry suggest that the amplitude of oesophageal contractions is higher in men than women, rises with increasing age and is higher in Afro-Caribbean than Hispanic and Caucasian populations(53, 65). This variation between demographic and racial groups may be due to specific effects of age, gender and race, or

common factors such as increased outflow resistance caused by central obesity. OH (weak, absent or failed peristalsis) is the most prevalent finding in clinical series. Smout et al reported that OH with or without hypotensive LOS was found in 58% of 2610 patients referred for (conventional) manometry(66). Other observers reported OH in 27–32% of patients presenting with non-obstructive dysphagia without GORD(67-70). Oesophageal hypocontractility is also the most prevalent oesophageal motor disorder in GORD, found in 21–38% of patients in large series(69, 71-73). In patients with GORD, OH is linked to the degree of mucosal damage expressed as oesophagitis. In a review by Kahrilas et al, peristaltic dysfunction was increasingly prevalent with increasing severity of peptic oesophagitis, occurring in 25% of patients with mild oesophagitis and 48% of patients with severe oesophagitis(74). Hence, patients with long segment Barrett's had a significantly lower distal oesophageal peristaltic amplitude as compared with normals (75). Other researchers reported that the majority of GORD patients diagnosed with OH have between 30% and 70% of their swallows followed by 'ineffective contractions', whereas a more severe form, with more than 80% of abnormal contractions, is less frequent and may represent 20–40% of all GORD patients with OH (4, 76).

Similarly a high prevalence of OH is found in patients with respiratory disorders and GORD (chronic cough, IPF, cystic fibrosis). OH was found in 53% of asthmatics, 41% of chronic coughers and 31% of those with laryngitis(3).

In **eosinophilic oesophagitis**, HRM shows frequently weak peristalsis and frequent failed peristalsis (77-83).

Most **connective tissue disorders** may affect oesophageal motility, either with impairment of the smooth muscle (scleroderma) or the striated muscle (dermato-polymyositis). Severe oesophageal dysmotility is most frequently observed in scleroderma. (84, 85). Scleroderma frequently involves the oesophagus, with severe gastroesophageal reflux (GER) and dysphagia as clinical consequences of oesophageal dysmotility. Castell et al studied 36 patients with scleroderma and reported that distal oesophageal aperistalsis was noted in 70% of patients. However, normal proximal oesophageal contraction pressures were documented in all cases(86). In a larger study, in 148 patients, decreased amplitude of the post-deglutition contraction-wave was seen in 79.8% patients(87).

4.1 Prevalence of OH in the Upper GI Physiology Unit (RLH)

In the upper GI Physiology at the Royal London Hospital (RLH) I receive more than 800 patients referred for oesophageal function assessment including different type of manometry and reflux monitoring. 32.5% of all referrals are OH patients out of which almost half have dysphagia (44%), and other symptoms included 57% reflux symptoms (i.e. heartburn and/or regurgitation), 23% cough. 1 in 3 of patients referred to this upper GI Physiology department has OH.

5 Pathogenesis of OH

The pathogenesis of OH is not completely understood. OH can be secondary to other diseases or as a primary entity.

5.1 Mechanism of OH

Data from experimental models of oesophagitis ((by inducing cycles of oesophagitis and healing),(88) in-vitro human tissue (studying effect of endogenous cytokines on oesophageal motor function)(89) and a positive response to prokinetic drugs suggest impaired cholinergic stimulation as the main defect underlying OH (90). Few myopathic pathologies can produce OH(91). OH can be observed in patients without GORD or connective tissue disorder. The pathogenesis of this idiopathic disorder (OH) is unknown, although Kim and coworkers(91) have provided initial evidence that an imbalance between the excitatory and inhibitory innervation of the oesophagus, reflected in the ratio between choline acetyltransferase (ChAT) and nitric oxide synthase (nNOS) expressed in the oesophageal muscle wall, may be present in patients with OH. This group recruited gastric cancer patients and reported that oesophageal tissues of patients with OH revealed histopathological changes of myopathy with a morphologically normal-appearing myenteric plexus, suggesting that the myopathic process may contribute to OH (91).

5.2 Causes of OH

The diseases that might lead to OH are listed in table 1-1.

Table 1-1 - Secondary causes of OH

Diabetes mellitus

- Autoimmune diseases
 - PSS - progressive systemic sclerosis
 - CREST syndrome
 - Sjogren's syndrome
 - Polymyositis-dematomyositis
 - MCTD – mixed connective tissue disease
- Neuro-muscular diseases
 - cerebrovascular (stroke), Parkinson's syndrome,
 - motoneuron-, demyelination diseases (MS)
 - muscular dystrophies, myasthenia gravis
- Chagas' disease - (Trypanosoma cruzi)
- Amyloidosis
- Presbyoesophagus
- Pharmacological agents ie anticholinergic medications

Scleroderma – There are three stages in the development of oesophageal involvement in scleroderma: neuropathy, myopathy, and fibrosis(92). The hallmark of the first stage is neural dysfunction due to arteriolar changes in the vasa nervorum. At this point, the smooth muscle may contract with methacholine, which acts directly on the muscle, but not with edrophonium, which enhances the effect of available acetylcholine by inhibiting its breakdown. The consequent

muscle ischemia characterises the second stage, leading to atrophy of the muscle layers. Finally, the muscle tissue is replaced by fibrosis(93) which then eliminates the response to methacholine. In patients with systemic sclerosis, oesophageal wall thickness has been reported to be no more than 3 mm(94). Ultrasound images show hyperechoic areas within the normal hypo-echoic muscularis propria corresponding to fibrosis found on histological sections of the autopsy specimens(95). The smooth muscle in the oesophagus is most commonly affected, provoking feeble contractions in the mid and distal oesophageal body and low LOS pressure. The striated muscle in the oesophagus is less frequently affected.

Severe hypomotility in the oesophageal body and low LOS pressure promote increased gastro-oesophageal reflux and impaired oesophageal clearance, particularly in the supine position(96-98). Consequently, oesophagitis and its complications (ulcer, stenosis and Barrett's oesophagus) are frequently observed in scleroderma. This may trigger a vicious circle which exacerbates OH. Both OH and reflux may contribute to pulmonary disease by micro-aspiration of acid and by vagal stimulation from oesophageal acid causing bronchoconstriction. Oesophageal manometric abnormalities were found to be more prevalent in patients with poor lung function and the most interstitial lung disease. Nevertheless, the causative role of oesophageal involvement in scleroderma related interstitial lung disease remains an area of debate(99). In general, OH in scleroderma is characterized histopathologically by atrophy and fibrosis of the muscular tissue and a normal-appearing myenteric plexus (100).

OH in GORD - Knowledge about the mechanisms underlying OH associated with GORD is accumulating. Acute (experimental) oesophagitis significantly reduces the frequency and amplitude of peristaltic contractions in the oesophagus (88, 101-103). In vitro studies suggested that acute oesophagitis dysmotility is mainly due to abnormal neural modulation (104, 105). Inflammatory mediators, such as interleukin-6 and platelet-activating factor, produced during acute oesophagitis, can diffuse through the oesophageal wall and reduce acetylcholine release from excitatory myenteric neurons to circular smooth muscle(89, 106, 107).

Experimental studies have shown that acute oesophagitis-associated with OH can disappear after spontaneous healing(88, 101, 103). In patients with chronic erosive GORD, however, healing of oesophagitis with medical or surgical treatment is not associated with complete recovery of oesophageal dysmotility(108-111), suggesting a secondary, irreversible motor abnormality or a primary phenomenon leading to OH. Nevertheless, it is uncertain whether OH associated with GORD is always the consequence of inflammation. It is also possible that it is a primary motor disorder leading to GORD(112).

The oesophageal body is a major component of the antireflux mechanism. Once reflux has occurred the reflux contents can be cleared by peristaltic sequences (113). An intact peristaltic mechanism is essential for effective acid clearance. Thus, disruption of oesophageal peristalsis affects clearance of the refluxates, resulting in excessive acid reflux and then onset of GORD (114).

It has been suggested that imbalance of excitatory and inhibitory mechanisms can be important in the pathogenesis of GORD related OH (91, 115). In GORD patients, ineffective oesophageal motility (IOM), is the most common motor abnormality (76). OH patients have more than the normal number of NOS-positive neurons in the circular muscle in the oesophagus, which might result in enhancement of inhibitory neural components (76).

5.3 Excessive activation of the local inhibitory neural reflex in onset of GORD

Application of capsaicin can attenuate the mechanical activity of the oesophageal striated muscle via activation of the local neural reflex including primary afferents and intrinsic neurons *in vitro* (41). In the mouse oesophagus, capsaicin inhibits the vagally mediated striated muscle contractions mainly through its action on mucosal primary afferents, which in turn activate the presumed inhibitory local reflex arc thus being involved in dysmotility of the oesophagus and then the pathogenesis of GORD. Acid exposure not only induces inflammation in the oesophageal mucosa (117) but also might influence afferent neurons expressing TRPV1, which can be stimulated by protons (118). If acid excessively activates local neural reflex expressing TRPV1 in the oesophageal body, oesophageal motility might be attenuated, resulting in decrease of clearance activity. In addition, functional changes of TRPV1 by pro-inflammatory mediators such as prostaglandin E2 (119) might facilitate activation of the inhibitory local neural reflex, resulting in low clearance activity. Therefore, it is presumed that excessive activation of the local inhibitory neural reflex might be involved in the pathophysiology of GORD.

Of course, dysmotility of the striated muscle portion of the oesophagus as described here might not directly be involved in gastroesophageal reflux in humans because the external muscle layer in the distal portion of human oesophagus is composed with smooth muscle fibers (37). Nevertheless, the above phenomenon might affect coordination of motility between striated muscle segment and the distal oesophageal motility contributing to OH . The inhibitory neural pathway activated by acid reflux has not been demonstrated in smooth muscle of the human oesophagus . In fact, spastic contractions are induced by acid reflux in the distal oesophagus (diffuse oesophageal spasm), which frequently are responsible for chest pain in GORD patients(120).

5.4 Abnormal relaxation of the LOS and GORD ⁽²⁾

Abnormal LOS function is important in GORD. This includes LOS hypotension and TLOSRS. LOS hypotension may be due to a number of potential disturbances, including abnormality of the muscle function itself, lack of normal cholinergic activation, decreased reflex excitation, decreased responsiveness to circulating substances such as gastrin, and activation of inhibitory system (121). The LOS is innervated by inhibitory and excitatory intrinsic neurons that are located in the myenteric plexus not only of the LOS but also of the oesophageal body. Abnormal activation of vagal afferents and/or efferents might activate inhibitory intrinsic neurons and cause LOS relaxation. It is reported that a subpopulation of myenteric nitrergic neurons is immunoreactive for a tachykinin receptor in the oesophageal body of a rat (122). Considering that myenteric neurons are closely innervated by spinal afferents in which TRPV1 and

tachykinins might be expressed in the oesophagus (37) as well as vagal afferent neurons, it is possible that acid can induce the release of tachykinins from afferent neurons and subsequently tachykinins would act on intrinsic nitrenergic neurons innervated to the LOS. This suggests that excessive acid reflux to the oesophageal body might evoke abnormal relaxation of the LOS by NO, resulting in severe GORD.

5.5 A putative vicious circle in onset and exacerbation of GORD (2)

Chronic oesophagitis may damage not only the mucosa but also intrinsic neurons (117). In fact, it has been reported that pro-inflammatory cytokines reduce oesophageal contraction by inhibiting release of acetylcholine from myenteric neurons (106). Oesophageal dysmotility might expose the mucosa to further acid exposure, which would cause more severe inflammation by directly influencing the mucosa or neurogenic mechanism via TRPV1-positive neurons and peptidergic neurotransmitters (123). Considering that the severity of myenteric plexus damage is positively correlated with the duration of history of oesophageal diseases (124), there might be a vicious circle in GORD (Figure 12).

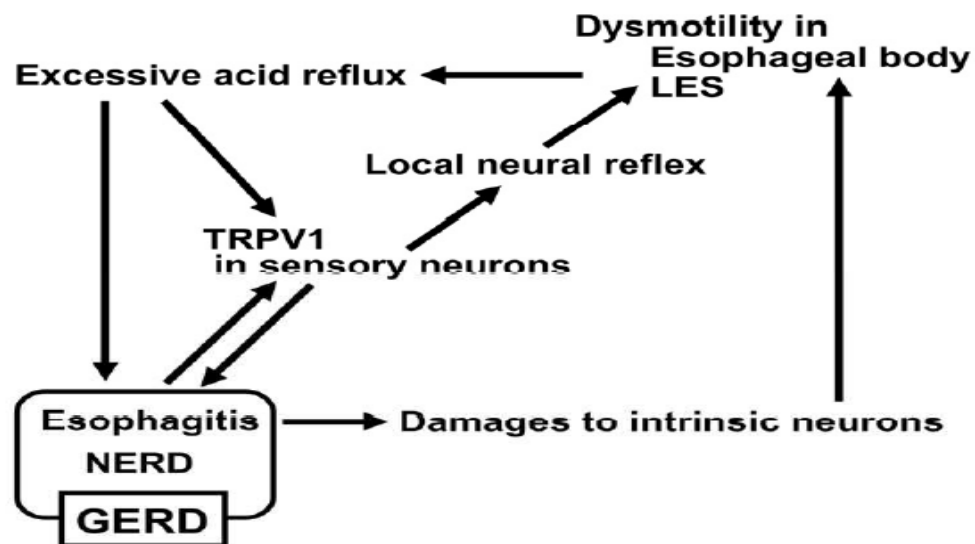


Figure 12 - A predicted vicious circle model of GORD. The circle might exacerbate GORD. GORD; gastroesophageal reflux disease. NERD; nonerosive reflux disease. LOS; lower oesophageal sphincter. TRPV1; transient receptor potential vanilloid 1. (Image source Shiina (2))

In general, inflammation affects nerves and muscle to alter LOS and oesophageal body motility. Both a decrease in cholinergic excitatory and an increase in nitrenergic and other inhibitory mechanisms appear to be involved. This combination would result in OH.

The decrease in cholinergic excitation may have a vagal component (125). Animal experiments and studies of human LOS tissue demonstrate a decrease in local cholinergic excitation (105, 126, 127), and major changes in calcium stores (128) can mediate oesophageal body contractility. In particular, prostanoids are involved. Inflammatory mediators such as interleukin-1B (IL-1B) and increases of reactive oxygen species (e.g. H₂O₂) are associated with increases in prostaglandin E₂ (PGE₂) and an isoform of PGE₂. Prostaglandin E₂ relaxes the LOS, whereas the

isoform of PGE₂ blocks prostaglandin F_{2a} (PGF_{2a})-mediated contraction (129). Prostaglandin E_{2a} along with thromboxane A₂/B₂ are important in maintenance of LOS tone, and blockade of PGE_{2a} activity further reduces LOS tone (130). Recent studies also indicate that inflammation induces the production of IL-6 in the mucosa and that IL-6, but not (IL-1B), leads to an increase of H₂O₂ in the muscle (131). H₂O₂ appears to be the main culprit that causes increases in platelet-activating factor (PAF) and PGE₂, both of which can act to reduce both ACh release (132) and LOS muscle tone (133). Earlier studies indicated that inhibition of prostaglandin synthesis with indomethacin prevented or corrected oesophagitis-associated LOS hypotension, presumably through a reduction of PGE₂ (134). oesophageal IL-8 is also increased in reflux oesophagitis, and presumably enhances neutrophil trafficking (135).

In addition to prostanoid effects, inflammation is associated with increased NO in oesophageal tissues (127) (136) and evidence of increased activity of the nitrergic inhibitory innervation (105, 127). These changes also result in low LOS pressure and decreased oesophageal body motility.

Of interest, acid infusion causes shortening of the oesophagus (137) in response in part to inflammatory mediators (138), and NO contracts longitudinal oesophageal smooth muscle (139). These responses to acid and acid-induced inflammation have been proposed as potential factors contributing to the development of hiatus hernia.

5.6 Relationship between OH and GORD

In spite of the high prevalence of both OH and GORD, the relation between the two remains controversial. OH has been blamed as the cause of abnormal oesophageal clearance and increased acid exposure, extra-oesophageal symptoms and dysphagia both before and after antireflux surgery. In recent studies, Fornari et al analysed the association between different degrees of OH and prolongation of acid clearance and increased oesophageal acid exposure(140). The results showed that only severe OH is associated with longer oesophageal clearance and the highest acid exposure, mainly in supine periods.

A recent study compared oesophageal motility in patients with NERD (non-erosive reflux disease) and patients with ERD (erosive reflux disease). 70% of the patients with ERD failed to respond to the physiologic challenge of solid bolus and MWS (multiple water swallows). This failure might result in impaired clearance following reflux events and increase exposure to gastric refluxate(141). A recent study showed that there is a higher prevalence of partial failure of peristalsis (segmental failure) compared to total failure of peristalsis (failed sequences) in subjects with reflux and Barrett's oesophagus (142). In this study fragmented smooth muscle contraction segments are considered to be a marker of OH .

In a different approach to study the OH in GORD, Kim et al(143),found a statistically significant increase in oesophageal-wall thickness, by using HFIUS, in patients with non-GORD-related ineffective oesophageal motility, when compared with controls, and with the patients who had both GORD and ineffective oesophageal motility. The investigators postulated that the pathophysiologic mechanisms that underlie ineffective oesophageal motility are different in patients with GORD, in whom the manometric abnormality may be induced by chronic acid-reflux exposure. In those patients without GORD, there may be a primary oesophageal muscular

disorder measured by HFIUS. This seems to correlate with a study by Mittal(144) of increased oesophageal wall thickness by HFIUS in patients with a number of oesophageal motor disorders(145).

6 Clinical Presentation of OH

Oesophageal symptoms in impaired oesophageal peristalsis include dysphagia, odynophagia, heartburn and regurgitation. Also, extra-oesophageal symptoms such as cough(146), globus and hoarseness are attributed to OH. However, the correlation between the severity of the manometric findings and symptoms is extremely poor. Even in patients with complete absence of peristalsis, as is often the case in scleroderma, symptoms may be absent. On the other end of the spectrum, one can find patients who complain of severe dysphagia but who have completely normal oesophageal peristalsis, LOS function, and bolus transit on barium studies.

Dysphagia is a common symptom in patients with oesophageal hypotensive peristalsis (147) and studies have shown that oesophageal clearance is compromised significantly when the amplitude of peristaltic contractions in the distal oesophagus falls to values below 25-30 mm Hg(58, 56).

Hypomotility in the oesophageal body impairs the refluxate clearance leading to prolonged oesophageal exposure to aggressive refluxate and GORD symptoms(56, 54). However, only severe OH is associated with prolonged clearance and acid exposure, particularly in supine periods(151).

The association of cough with OH is not clear at this moment and in fact this association might be independent from GORD in this group of patients. OH is more common in patients with chronic cough although many of them have a normal pH profile(146). The mechanism of cough in these patients is probably due to disordered peristalsis that may lead to impaired oesophageal clearance, as has been reported in a proportion of patients with chronic cough(152). This could result in prolonged stimulation of oesophageal cough receptors or micro-aspiration of oesophageal contents(153) causing direct stimulation of laryngeal and tracheal cough receptors.

7 Investigations

Symptom based diagnosis is not reliable in patients with swallowing problems, heartburn and other dyspeptic complaints (154, 155). The aim of investigation is to provide clinically relevant measurements of gastrointestinal (GI) structure and function that explain the cause of symptoms, identify pathology, and guide effective management(61, 157).

Endoscopic examination of the oesophagus is not a valuable tool to diagnose oesophageal motility although endoscopy should always be carried out to exclude ulceration, stenosis, and neoplastic lesions before the patient is referred for evaluation of oesophageal function. In OH oesophagoscopy is either normal or shows evidence of reflux oesophagitis.

Barium oesophagogram is a useful technique in the work-up of patients with a suspected oesophageal motility disorder. It will detect obstructive lesions, oesophageal dilation, and hiatus hernia as a complementary tool to endoscopy. In addition, and most importantly, the barium

oesophagogram provides information about oesophageal transit. For this purpose, not only barium suspension should be used, but also swallowing a solid bolus, such as a marshmallow or a piece of bread, should be part of the examination.

Scintigraphy does not provide structural information but is one of the best techniques (besides timed Barium swallow) that quantify oesophageal transit. Oesophageal scintigraphy can detect oesophageal involvement in patients with asymptomatic scleroderma, showing a typical pattern of retention of radioactivity in the lower oesophagus, with clearing after the patient is upright or drinks a glass of water. As an indicator of dysmotility in both early and advanced disease, oesophageal scintigraphy has a higher sensitivity than manometry and barium swallows(158).

Manometry is often considered to be the gold standard, being able to detect subtle impairment of oesophageal peristalsis. The most characteristic finding, low-amplitude simultaneous waves, can be observed in connective tissue diseases, diabetes, amyloidosis, myxedema, multiple sclerosis, chronic idiopathic intestinal pseudo-obstruction, and in severe end-stage GORD without scleroderma.

Major evolution in manometric methodology has been the introduction of high-resolution manometry (HRM); the basic concept being that by vastly increasing the number of recording sites and decreasing the spacing between them, one can more completely define the intraluminal pressure environment, minimizing the impact of spatial gaps between recording sites.(64) In recent years, HRM is believed to be an essential tool for mechanistic studies of oesophageal function in research and in clinical practice. HRM has been even used to study the effects of pharmacological agents on different oesophageal segments(159, 160). In clinical practice, HRM

has largely replaced conventional manometry. First of all HRM is easy to perform. Secondly, HRM predicts abnormal bolus transport more accurately than conventional manometry(157). Lastly, not only is the diagnostic agreement between conventional and HRM high(161) but regardless of clinical value and availability of the conventional method, some publications also emphasize that clinically important pathologies (impaired OGJ relaxation, achalasia, distal oesophageal spasm, localized abnormality of peristalsis) can be detected by HRM more accurately(157, 161)A classification of oesophageal motility disorders based on pressure topography characteristics has been proposed by the Chicago group(162, 163).

Whether conventional or high-resolution manometry is used, care must be taken to avoid circumstances that can lead to a spurious diagnosis of OH. Examples of these are drugs that inhibit oesophageal contractions (anticholinergic agents and calcium channel blockers), failure to have an appropriate time interval between swallows, and inclusion of dry swallows. Additionally, the appropriate normal values must be applied depending on the examination position because contractile vigor decreases on moving from the supine to the upright position(164).

The combination of oesophageal manometry and intraluminal impedance measurement allows assessment of the functional impact of ineffective oesophageal contractions. In a study of 350 patients, it was found that one-third of patients with a manometric diagnosis of OH had 'effective' transit for both liquid and viscous swallows(165). Similar findings were reported by others, suggesting that the definition of weak peristalsis should include functional correlates(60, 166). HRM, only when combined with fluoroscopy or impedance, clarifies the relationship between dysmotility and bolus retention(60, 166). A key insight from studies that combine

oesophageal manometry with impedance is that oesophageal symptoms are rarely caused by dysmotility unless this is accompanied by bolus retention or reflux(165, 167).

7.1 Complementary investigations in studying OH

Despite the technical advances set out above, standard methodologies using HRM still fail to establish a definitive diagnosis that explains the cause of symptoms in many patients with swallowing problems or reflux(157, 160). None of the standard methods are able to distinguish the underlying cause of the OH i.e. structural versus neurological defects. Consequently, these methods of oesophageal assessment have not been able to predict the outcome of medical treatments in these patients. Preoperative oesophageal manometry has not been able to distinguish the patients who may develop dysphagia after antireflux surgery (168) and so far there is no preoperative test to predict postoperative dysphagia in this group of patients (169).

These shortcomings might be due to the fact that HRM does not provide a direct assessment of oesophageal shortening, sensitivity, motor reserved capacity, or other biomechanical properties. Alternatively it may be because tests based on small volume water swallows in the supine position are not representative of normal behavior and/or do not ‘challenge’ oesophageal function.

7.1.1 *Additional analysis*

In recent years, attempts have been made to improve the sensitivity and the usefulness of oesophageal HRM testing by adding different parameters in the analysis of the oesophageal motility tracings. Pandolfino et al. defined the **flow permissive time** as the time when the bolus

domain pressure exceeds the OGJ obstruction pressure(170). A flow permissive time less than or equal to 2.5seconds had high sensitivity and specificity (86 and 92% respectively) for predicting incomplete oesophageal clearance. Incomplete bolus transit (IBT) is defined when bolus exit on impedance recording is not identified at any one of the three distal impedance-measuring sites. Normal transit is defined when 80% liquid and 70% viscous swallows demonstrates complete transit. IBT seems a reasonable surrogate end point for gauging the adequacy of peristalsis. IBT occurs more frequently with weak peristalsis. Kahrilas *et al.* (56) reported that IBT invariably occurred in the distal oesophagus when peristaltic amplitude was < 20 mm Hg, whereas it rarely occurred when the peristaltic amplitude was 31–45 mm Hg. Moreover, IBT is associated with dysphagia. Consistent with previous investigations (157, 171, 172), Roman et al(60) reported that failed peristaltic contractions and oesophageal pressure topography plots with breaks in the 20 mm Hg isobaric contour were associated with IBT. HRM plots with breaks > 5 cm (large) were consistently associated with IBT; 2–5 cm (small) breaks were variably associated with IBT. Topography plots without breaks or with breaks in the 20 mm Hg isobaric contour < 2 cm in length uniformly achieved complete bolus transit. Large (> 5 cm) and small (2–5 cm) pressure troughs in the 20 mm Hg isobaric contour of peristalsis, but not failed peristalsis (failed peristalsis = when the peristalsis is <3cm in length or <100mmHg.cm.s DCI), occurred more frequently in patients with unexplained non-obstructive dysphagia than in control subjects. The individuals with absent peristalsis (100 % failed peristalsis) were excluded from the latter study.

7.1.2

Additional methods

The use of provocative testing with solid or high volume water swallow challenge in correlation with symptom assessment have been shown to improve the diagnostic yield of HRM studies(173). Solid bolus swallows, multiple rapid swallows, and abdominal compression test are the provocative tests of choice applied in this study. The details of these tests are described in the following chapters in more detail.

In 2007, Lever *et al.*(174) introduced the possibility that an effortful swallow (i.e., volitional manipulation of the oropharyngeal phase of swallowing) may affect oesophageal peristalsis. The effortful swallow, which requires the patient to ‘swallow hard’, is often used as a treatment for oropharyngeal dysphagia. The authors reported increased amplitudes in the distal oesophagus during the effortful swallow compared with non-effortful swallowing. In 2012, Nekl et al reported that the effortful swallow condition yielded significantly higher oesophageal amplitudes across all sensor locations ($P < 0.05$). They also found that the effortful swallowing decreased the risk of incomplete bolus clearance when compared with non-effortful swallowing (OR: 0.51; 95% CI: 0.30–0.86)(175). However, there has not been much subsequent interest shown in the study of effortful swallowing in the literature and confirming the findings of the aforementioned authors requires further studies.

Most oesophageal motility studies focusing on oesophageal motility triggered by mechanical stimuli ie bolus volume rather than sensory factors such as chemothermal triggers of oesophageal motility. The use of carbonated water and different bolus temperatures on swallows have been studied in oropharyngeal motility with more significant results than on oesophageal body motility(176-178). Nevertheless, a recent study reported that chemothermal stimulation with

carbonation and cold boluses are most effective at modulating oesophageal body contractility(179). There are not many recent studies done on this matter however older studies confirm a similar effect of bolus temperature on oesophageal motility. Segall et al(180) reported that the mean amplitude of oesophageal contractions in response to cold (22°C) tap water swallows was 188 mm Hg (95% confidence interval, 165-211); In response to hot (60°C) swallows, the mean amplitude decreased to 125 mm Hg (95% confidence interval 106-144; $p < 0.001$). Other papers performed with standard oesophageal manometry reported that in healthy subjects oesophageal cooling decreased amplitude and velocity and increased duration of the peristaltic wave (338, 339). Overall, due to controversial findings regarding the effect of bolus temperature on oesophageal motility, some not showing effect on oesophageal body and some do and also regarding stimulating effect of hot or cold water, it requires further studies to decide whether this type of swallows can be used as clinical tool in oesophageal motility assessments(181).

7.2 Critical review of high resolution manometry (HRM)

HRM is a reproducible method for studying oesophageal motor disorders and is being accepted internationally as the physiological test of choice. However, considerable day-to-day variability may occur that should be taken into account when borderline findings are made during HRM (182). Furthermore, the ability of HRM to assess the OGJ pressure morphology such as radial and axial pressure effects is still limited (183). This will affect quantifying the indications for, and objectives of, antireflux surgery as well as OGJ bolus transit assessments.

Other limitations of HRM to consider:

- LOS pressure, TLOSRS, oesophageal body hypomotility and their relation to GORD has not been considered in the classification of oesophageal disorders using HRM
- There are no data on UOS and upper oesophageal motility disorders
- Rumination/postprandial belching cannot be identified, or is poorly defined in the current diagnostic criteria of HRM
- Normal values are only derived from 1 manufacturer of the HRM machines. Therefore, the data derived in one study with one brand of software and hardware cannot be generalized to other brands until more data are available (184).
- Effect of respiration on measuring amplitude of peristalsis has not been considered
- There is no established method in HRM to distinguish neurological versus muscular abnormalities in oesophagus. For example one cannot decide whether the hypomotility seen in a patient is due to muscular fibrosis or damage to excitatory neurons. Hence, reserved oesophageal motor capacity is not assessed.
- HRM provides minimal information about longitudinal muscle function
- There is no information regarding the luminal diameter of the oesophagus
- HRM alone cannot determine successful bolus transit hence recently combined HRM-impedance monitoring has been introduced
- HRM cannot provide any information regarding distensibility of the oesophagus and specifically OGJ. Lack of distensibility might explain the mechanism of having high intrabolus pressure in spite of complete relaxation of LOS.

7.3 EndoFLIP System

The endoluminal functional lumen imaging probe (EndoFLIP) system) (Crospon, Galway, Ireland) uses impedance planimetry for the real time measurement of the diameter of the oesophago-gastric junction. This system is the first to permit GOJ diameter to be directly measured without providing information about oesophageal body motility. Nevertheless, its clinical value is still under investigation and it is mostly used under general anaesthesia due to the amount of discomfort involved, especially during insertion.

8 Reversibility of OH

In the process of developing management strategies in OH, testing the potential reversibility of OH in patients could be useful to predict the response of these patients to new treatments and prokinetic drugs (reversible conditions may respond to prokinetic therapy). The reversibility of OH probably depends on the depth of injury within the oesophageal wall, the involvement of peripheral neural control of motility and/or oesophageal muscle layers, the inflammatory mechanism that is triggered ie due to oesophagitis, and the type of healing or restitution process. For example, patients with scleroderma have a severe defect in peristalsis due to replacement of oesophageal muscle with fibrous connective tissue which is not expected to be reversible(95), whereas patients with moderate oesophagitis may have OH that reverses after appropriate cholinergic stimulation with edrophonium (185).

Data from experimental acute oesophagitis (88, 186) and indirect evidence from acute positive response to prokinetic drugs in humans suggested an impaired cholinergic stimulation as the main defect (90).

Animal studies by Sifrim et al showed that repeated episodes of acute experimental oesophagitis lead to a progressive irreversible impairment of oesophageal motor function similar to that observed in patients with severe GORD (88). Another study on patients with GORD did not find any oesophageal motility improvement with meticulous antireflux therapy (111). In this study, it was concluded that impaired motility in reflux oesophagitis is either an irreversible consequence of oesophageal inflammation, or a (pre-existent) factor in its pathogenesis.

All standard methods of assessing oesophageal motility including high resolution manometry failed to provide information regarding potential reversibility of oesophageal motility. None of the standard methods have been able to accurately measure viability of oesophageal neuromuscular structure in patients with OH (non-standard methods which are not suitable for daily practice such as edrophonium test can be useful in non-clinical settings).

9 Treatment of OH

Specific treatment is clearly desirable for patients with evidence of symptoms related to OH. Effective control of acid reflux, if present, is the mainstay of clinical management at present.

9.1.1 *Dietary and lifestyle management*

A 'common sense' approach can reduce the risk of symptomatic bolus retention. Patients should favor liquid and semi-solid nutrition over solids, consume meals in the upright position, chew well and take plenty of fluids as these measures all promote oesophageal clearance (187). Indeed, it appears that the 'pharyngeal pump' together with gravity and hydrostatic forces can move not only liquids but also most solid food through the oesophagus without the need for active oesophageal contraction (140, 187). Many experts also recommend liberal use of carbonated beverages, because this may prevent as well as resolve bolus retention (188).

9.1.2 *Treatment of gastroesophageal reflux disease associated with hypotensive dysmotility*

OH patients with weak lower oesophageal sphincter function often experience severe symptoms and complications of GORD because poor clearance leads to prolonged acid exposure, particularly at night (92). These problems are marked in patients with systemic sclerosis in whom the combination of poor motility and poor salivation impacts on both volume and chemical (i.e. acid) clearance (93) although the relation of OH and GORD is still controversial and a place of debate. Dietary and lifestyle measures may be helpful in GORD and GORD

related OH, although these are rarely sufficient in severe GORD. A systematic review identified several such interventions that reduce oesophageal acid exposure(189), some of which may be of particular benefit in patients with hypotensive dysmotility (190, 191). These include (i) weight loss, (ii) keeping the upper body in an elevated position after a meal, (iii) lying down in the right lateral position, (iv) not smoking, (v) not consuming alcohol, (vi) reduction of meal size, and (vii) reduction in calorie load. Reduction in fat intake may be of additional value as this has high caloric density and also appears to sensitize the oesophagus to acid reflux events (192). In addition, chewing gum for half an hour after meals may be helpful(193), as this stimulates salivation and swallowing, improving both volume and chemical clearance.

High-dose acid suppression taken twice a day is often required to suppress gastric acid, heal oesophagitis and provide effective symptom relief in patients with severe hypotensive disease(194). Some patients benefit also from alginate preparations taken after a meal to suppress both acid and non-acid reflux events by forming a viscous layer over the gastric contents(194). The addition of ranitidine to suppress basal nocturnal acid secretion appears to be helpful in some patients but was not effective in a randomized controlled trial in 14 patients with systemic sclerosis (195).

9.1.3 *Prokinetics*

Cholinergic agents Medications that increase the concentration of acetylcholine in the synaptic cleft or directly stimulate muscarinic receptors promote smooth-muscle contractility. Bethanechol, a direct-acting muscarinic receptor agonist, has been shown in healthy volunteers and patients with hypotensive oesophageal dysmotility to increase peristaltic amplitude in the

distal-oesophagus(196). Using combined multichannel intraluminal impedance- manometry in seven patients with severe OH, Agrawal and coworkers(196) demonstrated that a single oral dose of 50 mg bethanechol increased both contractile pressure and bolus clearance. Similar effects on contractile pressure were reported by Blonski and coworkers(197) for a range of oral cholinergic agents, including bethanechol (25 mg), pyridostigmine (60 mg), and buspirone (20 mg), with pyridostigmine also promoting bolus transport. No trials demonstrating clinical efficacy have been published. Nevertheless, some experts report benefit of these medications in individual patients, although side-effects such as excessive salivation and diarrhea may limit their use.

9.1.3.1 Dopamine antagonists

Domperidone is a D₂ receptor (dopaminergic receptor type 2) antagonist that promotes gastrointestinal motility by antagonizing the inhibitory effects of dopamine on postsynaptic cholinergic neurons in the myenteric plexus(198). Metoclopramide augments this peripheral effect with procholinergic properties and also has central anti-emetic actions at the chemoreceptor trigger zone(199). These medications increase LOS pressure, accelerate gastric emptying and improve symptoms in patients with GORD and also diabetic gastroparesis(200, 201). Effects on oesophageal peristalsis and clearance are well established. No effect of 20 mg domperidone on oesophageal emptying is found on scintigraphy in 12 patients with diabetic autonomic neuropathy and oesophageal dysfunction(202). In contrast, a significant improvement in clearance was reported after administration of 10 mg intravenous metoclopramide in 14 patients with systemic sclerosis(203). MHRA guidelines suggest that Domperidone should not be used when stimulation of gastric motility could be harmful: gastro-intestinal haemorrhage,

mechanical obstruction or perforation (MHRA publication on “DOMPERIDONE 10MG TABLETS”, PL 21880/0110, UKPAR).

9.1.3.2 Motilin agonists and Azithromycin

Erythromycin and other macrolide antibiotics have pronounced prokinetic effects that are utilized by physicians treating patients with severe gastrointestinal dysmotility such as gastroparesis and pseudo-obstruction(204). This effect is mediated by motilin receptors that play a key role in the initiation of phase III migrating motor complex (MMC), inter-digestive ‘housekeeping’ contractions that sweep the stomach, and bowel clear of undigested material and bacterial overgrowth(204). Chrysos and co-workers (205), (206) showed that intravenous erythromycin (200 mg i.v. bolus) increased contractile vigor and LOS pressure in 15 GORD patients. Erythromycin (200/500 mg) also given intravenously reduced post-prandial gastroesophageal reflux in GORD patients by 50% (207, 208). In a 2-week clinical study Chang and co-workers(209) reported that erythromycin (250 mg tid) significantly shortened oesophageal and gastric transit and improved glycaemic control in diabetic patients. Similarly, other researchers reported that erythromycin improved oesophageal transit in patients with diabetes and autonomic dysfunction (67, 210). Although these findings are significant, the clinical use of erythromycin is limited by tachyphylaxis and side-effects including dyspepsia and diarrhoea. Erythromycin used in adults and paediatric patients with OH (211) showed variable and somewhat disappointing results. New motilin agonists that may be better tolerated are in

development. However, one recent example, ABT-229, had no effect on LOS function, oesophageal motility and reflux in GORD patients(212).

Recently, Mertens *et al* reported that the macrolide azithromycin (AZI), a macrolide similar in structure and function to erythromycin (213), reduced the rate of reflux episodes in patients with a lung transplant (214, 215). Boeckxtaens *et al* showed that during treatment with AZI, the proximal extent of refluxate was significantly reduced compared with placebo. In patients with small HH, treatment with AZI led to a significantly smaller mean hiatal hernia size before reflux episodes. In line with this, the hiatal hernia was more often in the reduced state during AZI treatment than during placebo. Boeckxtaens concluded that the effect of azithromycin on reflux was mainly caused by a more distal position of the acid pocket, probably resulting from a reduction of the hiatal hernia size. An alternative hypothesis is that azithromycin accelerates gastric emptying and improves mixing of stomach contents, potentially affecting acid pocket properties(216, 217). Further reduction in acid secretion by AZI might have contributed to the reduced number of acidic reflux events. Yet another hypothesis is that azithromycin improves oesophageal motility which in turn reduces the clearance time seen in those patients. Other studies on the effect of azithromycin on gastrointestinal motility showed effect of this drug on the gastric antrum and duodenum to be stronger than that of erythromycin with longer duration of effect (218, 219).

9.1.3.3

5 HT agonists

Cisapride and mosapride are prokinetic agents with mixed 5-HT₄ agonist/5-HT₃ antagonist action. Tegaserod, prucalopride, and other selective 5HT₄ agonists have similar actions(199). Serotonin is released from enterochromaffin cells on mechanical stimulation and 5-HT₄ receptors facilitate acetylcholine release in the myenteric plexus that triggers peristaltic contraction and clearance(220). Thus, in contrast to muscarinic antagonists and motilin agonists, 5-HT₄ agonists promote normal gastrointestinal transit rather than inducing powerful but un-physiological contractions. These agents have prokinetic effects throughout the gastrointestinal tract and proven clinical efficacy in various conditions characterized by slow-transit, including GORD, diabetic gastroparesis and constipation(221). Studies have demonstrated that cisapride and mosapride increase LOS pressure, promote oesophageal clearance, and reduce acid exposure in health and GORD patients(222, 223). However, the mechanism of this action was not evident on conventional motility studies(222-224). Soon after the introduction of high-resolution manometry with oesophageal pressure topography Staiano and Clouse(225) observed that cisapride enhanced contraction in the proximal smooth-muscle segment of the oesophageal body. The functional significance of this effect was confirmed by combined HRM-videofluoroscopy that showed tegaserod improved co-ordination between contractile segments, leading to more effective solid-bolus transport (Figure 12)(159). Cisapride and tegaserod have been withdrawn due to rare, but occasionally life-threatening, side-effects; however, new 5-HT₄ agonists are in the pipeline or are in the market approved for other indications(221). Clinical trials in GORD are in progress and, hopefully, studies in symptomatic, hypotensive oesophageal motility will follow.

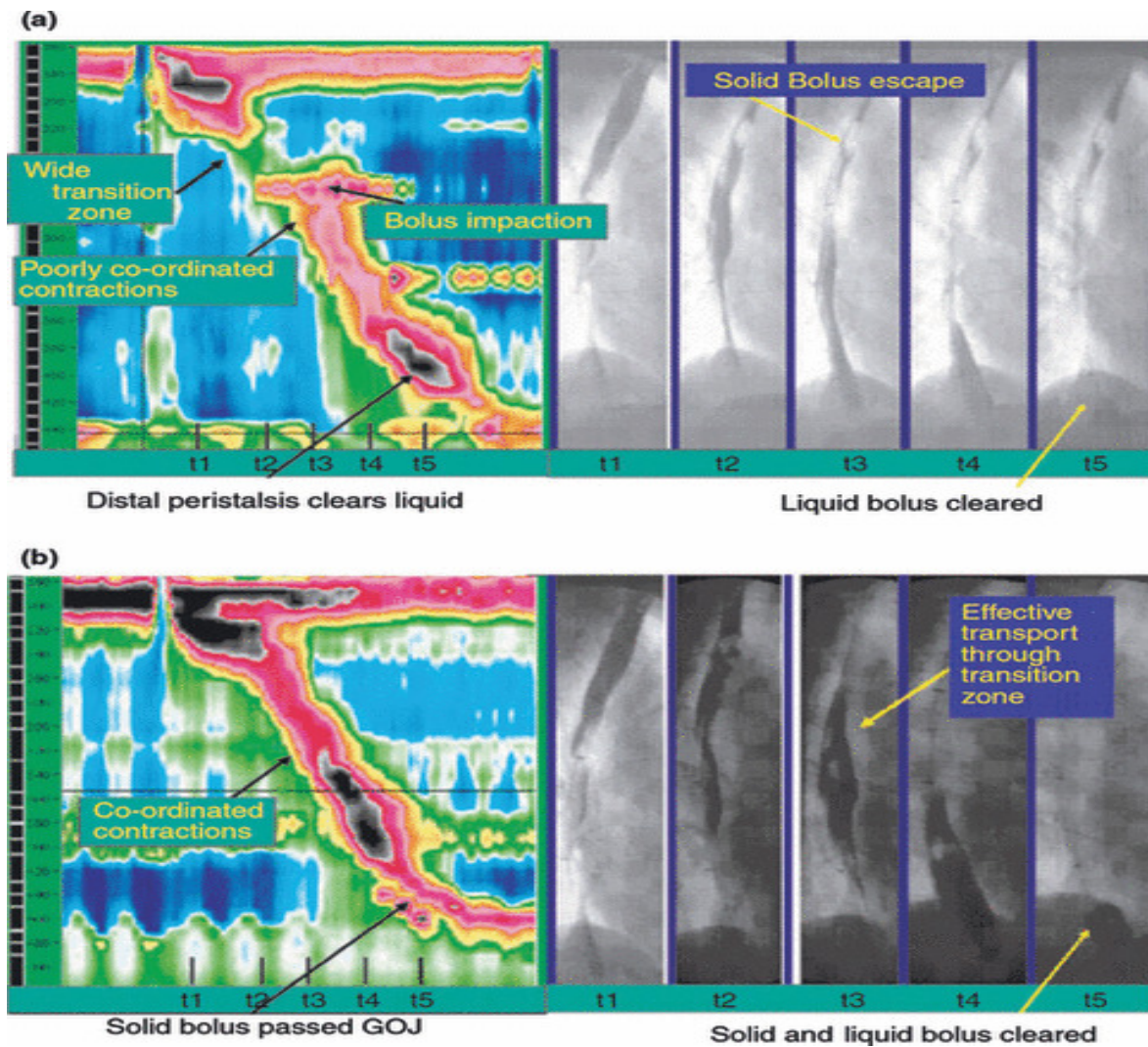


Figure 13 - Concurrent fluoroscopy and high-resolution manometry (HRM) reveals the functional importance of co-ordination between the proximal and mid-distal oesophageal contractions for solid-bolus transport and the prokinetic effects of the 5-HT₄ agonist tegaserod. (A) Patient no. 6: placebo treatment. HRM shows a break in the contractile front (>3 cm) at the proximal transition zone, the peristaltic contraction is otherwise preserved. Concurrent fluoroscopy reveals solid-bolus escape at the level of the proximal transition zone (note the corresponding pressure rise at the level of bolus impaction). In contrast, the liquid barium ingested with the marshmallow was propelled into the distal-oesophagus and most was transported into the stomach. (B) Patient no. 6: tegaserod treatment. The pressure trough at the proximal transition zone is less pronounced on the HRM plot, the peristaltic contraction in the proximal oesophagus is well co-ordinated with the mid- and distal-oesophagus. Concurrent fluoroscopy reveals effective solid and liquid bolus transport (note the pressure rise as the bolus passes through the gastro-oesophageal junction into the stomach). Adapted with permission from Fox et al. *Aliment Pharmacol Ther* 2006; 24: 1017–1027.

9.1.4

Surgery

In patients with severe GORD, impaired peristalsis, impaired oesophageal clearance, and dysphagia are common. The dysphagia may not only be due to the hypotensive dysmotility(56), but also to mechanical outflow obstruction at the oesophagogastric junction in the presence of hiatus hernia(226). In some cases anti-reflux surgery may not only improve reflux symptoms but also reduce dysphagia(108, 227-231). This may be due to improvement of oesophageal motility and visceral hypersensitivity with normalization of acid exposure or due to reduction of the hiatus hernia. However, the literature on the effect of fundoplication on oesophageal motility and the relationship between preoperative motility and outcome of surgery should be interpreted with caution. Flaws in the design of these studies and manometric techniques employed should be taken into account. Although some of these studies concluded that hypotensive dysmotility is not a contra-indication to surgical management of GORD, many experts in the field hold the opinion that fundoplication should not be carried out in patients with severe OH.

9.2 Summary

OH is a term used to define low amplitude contractions in the body of oesophagus and hypotensive LOS and is frequently found in patients with non-obstructive dysphagia and in gastroesophageal reflux disease. Oesophageal manometry is considered the "gold standard" test for the evaluation of oesophageal motility. Compared to conventional manometry, High-resolution manometry (HRM); vastly increasing the number of recording sites and decreasing the

spacing between them, can more completely define the intraluminal pressure environment, by minimizing the impact of spatial gaps between recording sites.(2)

OH can be a secondary phenomenon associated with severe inflammation or systemic disorders such as scleroderma, but often occurs in patients without significant reflux disease or evident systemic disease. Clinically OH may present with symptoms of GORD (heartburn and/or regurgitation), dysphagia, extra-oesophageal symptoms such as cough and hoarseness and it may also develop after antireflux surgery (fundoplication).

In the assessment of OH, to exclude mechanical/pathological causes of dysphagia, upper endoscopy and barium swallow should be performed. Motility testing is performed by oesophageal HRM or preferably by combined HRM-impedance monitoring which can define both a detailed motor pattern and its impact on bolus transit and oesophageal emptying.

The presence of OH in experimental manometric studies with single liquid swallows might not reflect the status of oesophageal contractility during meals or after reflux in “real life”, Therefore, complementary stimulation tests could potentially add further information regarding the neuromuscular capacity of oesophageal wall.

To date, prokinetics have shown disappointing results in treatment of OH in which might be due to a failure to phenotype patients before treatment according to the reserved oesophageal neuromuscular capacity. It thus has the potential to be a new medication for the treatment of gastroparesis and gastrointestinal dysmotility.

9.3 Remaining questions and aim of this thesis

OH is a common clinical condition in patients with dysphagia (69). OH can be due to a functional neuromuscular disorder or to a structural change of the oesophageal anatomy (i.e. fibrosis, connective tissue disorder or infiltration). The former can theoretically improve with adequate stimulation whereas structural disorders can be irreversible. It could be, therefore, very important to assess the degree of reversibility of this condition for management purposes.

Several pharmaceutical agents known as prokinetic drugs can stimulate GI motility. Objective assessment of the severity of oesophago-gastric hypomotility and viability (as non-viable muscle will not react to therapies) of oesophageal neuromuscular function, prior to prokinetic therapy, would improve the clinical impact of these agents in patients with the appropriate phenotype.

Moreover, a diagnostic tool for patients with OH, able to predict response to prokinetic therapy is an unmet need. Oesophageal manometry provides the diagnosis of hypomotility but does not reveal the potential reversibility or severity of this condition.

9.4 Aims

- 1) To assess IOM in patients with non-obstructive dysphagia and GORD, using HRM;
- 2) To develop and standardize a set of “stimulation tests” to assess degree of IOM reversibility
- 3) To treat IOM with a prokinetic agent and assess the outcome on oesophageal motility, dysphagia and GORD;

- 4) To evaluate the role of stimulation tests in predicting manometric and clinical to prokinetic therapy in patients with IOM

9.5 How these aims are achieved in this PhD project

1. Studied healthy asymptomatic subjects to obtain normal values for oesophageal motility and “oesophageal stimulation tests”
2. Assessed reproducibility of “stimulation tests” in normal subjects
3. Assessed the relationship between the response to stimulation test and symptom characteristics in patients with IOM
4. Performed a randomized placebo controlled clinical trial to assess the effect of the prokinetic Azithromycin (AZI) on severe IOM in patients with dysphagia and/or GORD

CHAPTER 2:

SUBJECTS AND METHODS

CHAPTER 2: SUBJECTS AND METHODS

The methods and materials used in the studies presented in this PhD thesis will be described here, and specific methods will be presented in greater details in the relevant chapters.

10 Subjects

Healthy volunteers within the age range of 18-70 years old with no reflux symptoms, dysphagia, history of GI surgery or major medical conditions are studied to establish the normal values for the HRM device and stimulation tests used in this study.

Patients with non-obstructive dysphagia and GORD with diagnosis of IOM are studied at the upper GI physiology unit at the Royal London Hospital. These patients presented with dysphagia, gastro-oesophageal reflux symptoms, cough or a combination of all the three symptoms.

Recruitment Procedure

Healthy volunteers are recruited from the general public by advertisement and direct contacts.

The volunteers who met the inclusion criteria were contacted via letter or telephone and invited to participate.

All the patients undergoing oesophageal motility testing at the Upper GI Unit at the Royal London Hospital as well as at the same unit at Guy's Hospital with a diagnosis of severe OH were considered as potential participants in the relevant studies of this PhD project.

11 METHODS

11.1 Questionnaires

Standardized questionnaires were used to quantify symptoms and assess the effect of the interventions i.e. prokinetic/placebo treatment:

To evaluate the effect of AZI on symptoms I used the following questionnaires:

Dysphagia Odynophagia Questionnaire – This is a validated 10-item questionnaire that assesses the frequency of dysphagia, food impaction and odynophagia. Items are scored from 0-5, using a Likert scale where higher scores represent worse symptoms. A total score out of 50 is calculated – higher scores represent more severe dysphagia. A score of >5 has 86% sensitivity and 97% specificity for identifying the presence of objective dysphagia to avoid inaccurate description of

other symptoms such as feeling of fullness in epigastrium in place of dysphagia (Escobar, Pandolfino et al. 2011).

Reflux symptoms were assessed using the Reflux Disease Questionnaire (RDQ) (232) – This is a 12-item self-administered questionnaire, designed to assess the frequency and severity of heartburn, regurgitation, and dyspeptic complaints and to facilitate the diagnosis of GORD in primary care (232). It scores 12 individual items relating to the frequency and severity of reflux, using a Likert scale, where 0 represents the most negative option and 5 the most positive one. A raw score is calculated for domains of heartburn (score: 0-20), regurgitation (score: 0-20) and dyspepsia (score: 0-20), the scores of heartburn and regurgitation can be combined to give a total GORD score (0-40) (Since dyspepsia is not considered a typical GORD symptom it is eliminated to establish a specific GORD scoring when indicated).

Psychological assessment was performed using Hospital Anxiety and Depression Scale (HADS) (233). This rating scale has been established as a much applied and convenient self-rating instrument for anxiety and depression in patients with both somatic and mental problems, and with equally good sensitivity and specificity as other commonly used self-rating screening instruments. The HADS is a fourteen item scale that generates ordinal data. Seven of the items relate to anxiety and seven relate to depression. Each item on the questionnaire is scored from 0-3 and this means that a person can score between 0 and 21 for either anxiety or depression. The licence to use this questionnaire has been obtained for the Neurogastroenterology team at the site of conducting this project.

Dysphagia questionnaire

Mark only one answer for each item

Over the past 30 days, on average, how often have you had the following?

	Never	Less than once a month	1-9 times a month	10-19 times a month	20-29 times a month	Daily
a. trouble eating solid food (meat, bread)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b. trouble swallowing liquids	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
c. pain while swallowing	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
d. trouble eating soft foods (yogurt, jello, pudding)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
e. coughing or choking while swallowing foods	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Over the past 180 days (6 months), on average, how would you rate your discomfort or pain during swallowing?

	None	Very mild	Mild	Moderate	Moderately severe	Severe
a. eating solids (meat, bread)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b. eating soft foods (yogurt, jello, pudding)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
c. drinking liquids	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Over the past year (12 months), how often have you had the following?

	Never	1 time in the past year	2 times in the past year	3 times in the past year	4 times in the past year	More than 4 times in the past year
f. food stuck in throat or esophagus for more than 30 minutes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
g. an emergency room visit because of food being stuck in throat or esophagus	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Reflux Disease Questionnaire (RDQ)

Please answer each question by ticking one box per row.

1. Thinking about your symptoms over the past 7 days, how often did you have the following?

	Did not have	1 day	2 days	3-4 days	5-6 days	Daily
a. A burning feeling behind your breastbone	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b. Pain behind your breastbone	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
c. A burning feeling in the centre of the upper stomach	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
d. A pain in the centre of the upper stomach	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
e. An acid taste in your mouth	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
f. Unpleasant movement of material upwards from the stomach	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

2. Thinking about your symptoms over the past 7 days, how would you rate the following?

	Did not have	Very mild	Mild	Moderate	Moderately severe	Severe
a. A burning feeling behind your breastbone	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b. Pain behind your breastbone	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
c. A burning feeling in the centre of the upper stomach	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
d. A pain in the centre of the upper stomach	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
e. An acid taste in your mouth	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
f. Unpleasant movement of material upwards from the stomach	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Hospital Anxiety and Depression Scale (HADS)

The following two pages of questions are about how you have been feeling recently. By placing a tick in one box in each group below, please indicate which statement best describes how you have been feeling in the past week. Please do not tick more than one box for each question.

In the past week, ...

- C1 I feel tense or "wound up"
- | | | |
|---------------------------------|--------------------------|---|
| Most of the time | <input type="checkbox"/> | 1 |
| A lot of the time | <input type="checkbox"/> | 2 |
| From time to time, occasionally | <input type="checkbox"/> | 3 |
| Not at all | <input type="checkbox"/> | 4 |
- C2 I still enjoy things I used to enjoy
- | | | |
|--------------------|--------------------------|---|
| Definitely as much | <input type="checkbox"/> | 1 |
| Not quite as much | <input type="checkbox"/> | 2 |
| Only a little | <input type="checkbox"/> | 3 |
| Hardly at all | <input type="checkbox"/> | 4 |
- C3 I get a sort of frightened feeling as if something awful is about to happen
- | | | |
|-----------------------------------|--------------------------|---|
| Very definitely and quite badly | <input type="checkbox"/> | 1 |
| Yes, but not too badly | <input type="checkbox"/> | 2 |
| A little, but it doesn't worry me | <input type="checkbox"/> | 3 |
| Not at all | <input type="checkbox"/> | 4 |
- C4 I can laugh and see the funny side of things
- | | | |
|----------------------------|--------------------------|---|
| As much as I always could | <input type="checkbox"/> | 1 |
| Not quite so much now | <input type="checkbox"/> | 2 |
| Definitely not so much now | <input type="checkbox"/> | 3 |
| Not at all | <input type="checkbox"/> | 4 |
- C5 Worrying thoughts go through my mind
- | | | |
|--------------------------|--------------------------|---|
| A great deal of the time | <input type="checkbox"/> | 1 |
| A lot of the time | <input type="checkbox"/> | 2 |
| Not too often | <input type="checkbox"/> | 3 |
| Very little | <input type="checkbox"/> | 4 |
- C6 I feel cheerful
- | | | |
|------------------|--------------------------|---|
| Never | <input type="checkbox"/> | 1 |
| Not often | <input type="checkbox"/> | 2 |
| Sometimes | <input type="checkbox"/> | 3 |
| Most of the time | <input type="checkbox"/> | 4 |
- C7 I can sit at ease and feel relaxed
- | | | |
|------------|--------------------------|---|
| Definitely | <input type="checkbox"/> | 1 |
| Usually | <input type="checkbox"/> | 2 |
| Not often | <input type="checkbox"/> | 3 |
| Not at all | <input type="checkbox"/> | 4 |

- C8** I feel as if I am slowed down
- | | | |
|---------------------|--------------------------|---|
| Nearly all the time | <input type="checkbox"/> | 1 |
| Very often | <input type="checkbox"/> | 2 |
| Sometimes | <input type="checkbox"/> | 3 |
| Not at all | <input type="checkbox"/> | 4 |
- C9** I get a sort of frightened feeling like “butterflies” in the stomach
- | | | |
|--------------|--------------------------|---|
| Not at all | <input type="checkbox"/> | 1 |
| Occasionally | <input type="checkbox"/> | 2 |
| Quite often | <input type="checkbox"/> | 3 |
| Very often | <input type="checkbox"/> | 4 |
- C10** I have lost interest in my appearance
- | | | |
|---------------------------------------|--------------------------|---|
| Definitely | <input type="checkbox"/> | 1 |
| I don't take as much care as I should | <input type="checkbox"/> | 2 |
| I may not take quite as much care | <input type="checkbox"/> | 3 |
| I take just as much care as ever | <input type="checkbox"/> | 4 |
- C11** I feel restless as if I have to be on the move
- | | | |
|------------------|--------------------------|---|
| Very much indeed | <input type="checkbox"/> | 1 |
| Quite a lot | <input type="checkbox"/> | 2 |
| Not very much | <input type="checkbox"/> | 3 |
| Not at all | <input type="checkbox"/> | 4 |
- C12** I look forward with enjoyment to things
- | | | |
|--------------------------------|--------------------------|---|
| As much as I ever did | <input type="checkbox"/> | 1 |
| Rather less than I used to | <input type="checkbox"/> | 2 |
| Definitely less than I used to | <input type="checkbox"/> | 3 |
| Hardly at all | <input type="checkbox"/> | 4 |
- C13** I get sudden feelings of panic
- | | | |
|-------------------|--------------------------|---|
| Very often indeed | <input type="checkbox"/> | 1 |
| Quite often | <input type="checkbox"/> | 2 |
| Not very often | <input type="checkbox"/> | 3 |
| Not at all | <input type="checkbox"/> | 4 |
- C14** I can enjoy a good book or radio or television programme
- | | | |
|-------------|--------------------------|---|
| Often | <input type="checkbox"/> | 1 |
| Sometimes | <input type="checkbox"/> | 2 |
| Not often | <input type="checkbox"/> | 3 |
| Very seldom | <input type="checkbox"/> | 4 |

End of questionnaire

11.2 Oesophageal physiology tests

11.2.1 *High resolution manometry – Technique*

Manometry is often considered to be the gold standard, being able to detect subtle impairment of oesophageal peristalsis. A major evolution in manometric methodology has been the introduction of high-resolution manometry (HRM); the basic concept being that by vastly increasing the number of recording sites and decreasing the spacing between them, one can more completely define the intraluminal pressure environment, minimizing the impact of spatial gaps between recording sites (64). In recent years, HRM has become an essential tool for mechanistic studies of oesophageal function in research and in clinical practice (Figure 13). HRM has been even used to study the effects of pharmacological agents on different oesophageal segments(159, 160). In clinical practice, HRM should replace conventional manometry. First of all HRM is easy to perform and probably more reproducible. Secondly, HRM predicts abnormal bolus transport more accurately than conventional manometry(157). Lastly, not only is the diagnostic agreement between conventional and HRM high(161) but some publications also emphasize that clinically important pathologies (impaired OGJ relaxation, achalasia, distal oesophageal spasm, localized abnormality of peristalsis) can only be detected by HRM(157, 161)A classification of oesophageal motility disorders based on pressure topography characteristics has been proposed by the Chicago group (162, 163).

Whether conventional or high-resolution manometry is used, care must be taken to avoid circumstances that can lead to a spurious diagnosis of OH. Examples of these are the use of

drugs that inhibit oesophageal contractions (anticholinergic agents and calcium channel blockers), failure to have an appropriate time interval between swallows, and inclusion of dry swallows. Additionally, depending on the examination position, the appropriate normal values must be applied because contractile vigor decreases on moving from the supine to the upright position(164).

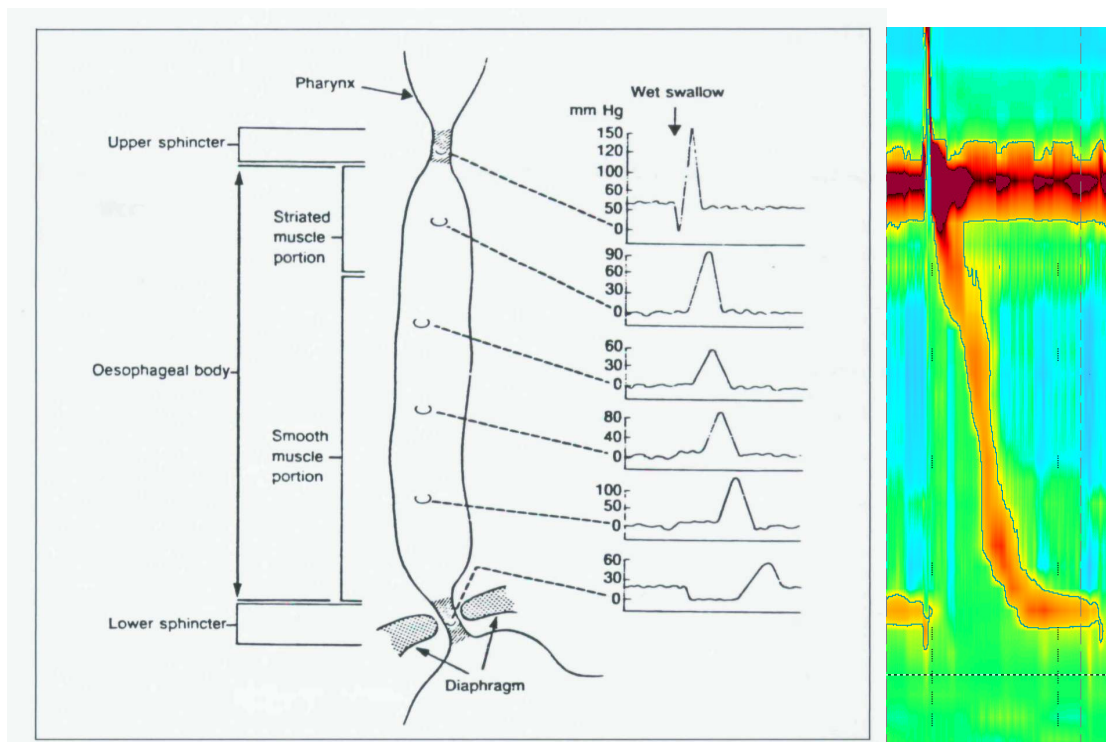


Figure 14 - Left: Station pull-through manometry courtesy of J. R. Siewert, H. Feussner (Munich); Right: high resolution manometry for a single swallow. (Image from internal source)

11.2.2 HRM Protocol

After overnight fasting, pressure calibration and applying local anaesthetic to nasal and pharyngeal cavities, a 32-channel solid state circumferential combined manometric and impedance monitoring catheter (Unisensor, AG) was inserted transnasally to 60 cm from the nares. The participant lay down on their right side and rested for 5 minutes before any measurements were taken. The gastric baseline was recorded and the catheter positioned in order that both upper and lower oesophageal sphincters were visible on the screen. Basal oesophageal body and LOS measurement were recorded. Using a syringe to inject water orally, a total of 10 single swallows of 5ml water was performed. There was at least a 20 second interval between each swallow.

Lying down position was preferred in this study as it increases the workload of the oesophagus making it more accurate (both sensitive and specific) to detect peristaltic dysfunctions(234).

After recording basal oesophageal motility with 10 water swallows, stimulation tests follow without any change to the HRM settings or the participant's position.

Stimulation tests

The first stimulation test consisted of three series of multiple rapid swallows (MRS). The participant swallowed in total 10mls of water in 5 swallows with 1-2 second intervals (each swallow contained 2mls of water).

The next stimulation test was an abdominal compression test. A specially designed inflatable cuff (21 x 55 cm) (VBM Medizintechnik, Sulz, Germany) was wrapped around the abdomen to

induce external abdominal compression which in turn will increase gastric pressure and oesophageal outlet resistance to bolus transit. The cuff is equipped with external manometers. The participant was given 10 swallows of 5mls of water with sufficient time intervals to allow oesophageal motility to settle back to baseline.

The last step, after 10-15 minutes rest, was solid bolus swallows. The participant swallowed 10 pieces (1-2 cm³)(164) of white bread without crust in 10 single swallows. Between each swallow the participant was given enough time to recover from each swallow.

11.3 Detailed description of oesophageal stimulation tests

11.3.1 Multiple rapid swallowing (MRS):

Multiple rapid swallowing (MRS), by overloading the oesophagus with water, provokes an intense central and peripheral oesophageal neural inhibition resulting in the absence of contractions in the smooth muscle portion of the oesophagus and prolonged, complete relaxation of the LOS. The last swallow of the MRS series is followed by a powerful peristaltic sequence in the oesophageal body together with a post relaxation contraction in the LOS (24). A normal response to multiple rapid swallowing requires integrity of inhibitory and excitatory mechanisms (regulating oesophageal body and LOS motility) as well as oesophageal muscle integrity. Multiple rapid swallowing could be an intense stimulation exercise to test the integrity of inhibitory and excitatory mechanisms that regulate oesophageal motility. I use MRS to test oesophageal and LOS neuromuscular viability in patients with hypomotility.

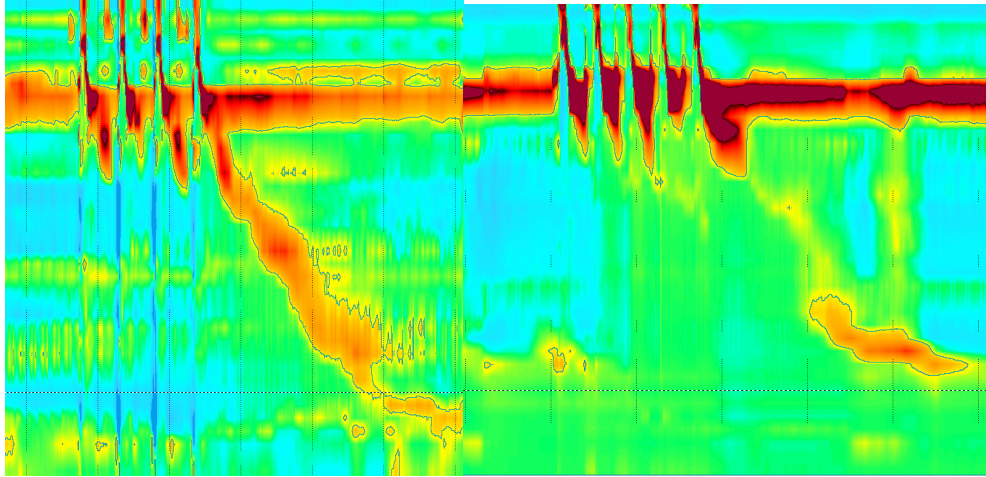


Figure 15 - Normal (left) and abnormal (right) response to MRS. (Image from internal source)

11.3.2 *Solid food swallows:*

Studies have shown that swallowing bread boluses can produce peristalsis with higher amplitude, longer duration and slower velocity than single water swallows(25-26). Peristaltic velocity becomes progressively slower and contractile pressure more powerful as the work required for bolus transport increases from dry (i.e.; no bolus) to fluid swallows and from fluid to solid bolus swallows (27). The contractile integral, a variable that summarizes the vigour of oesophageal contractility (2), is higher in the smooth muscle oesophagus during solid rather than liquid bolus transport and the pressure gradient across the gastro-oesophageal junction is greater. (28). Therefore, it is considered that resistance to solid bolus transit would provide a greater challenge to oesophageal function (28). I use solid bolus swallows as part of testing oesophageal and LOS neuromuscular viability in patients with hypomotility.

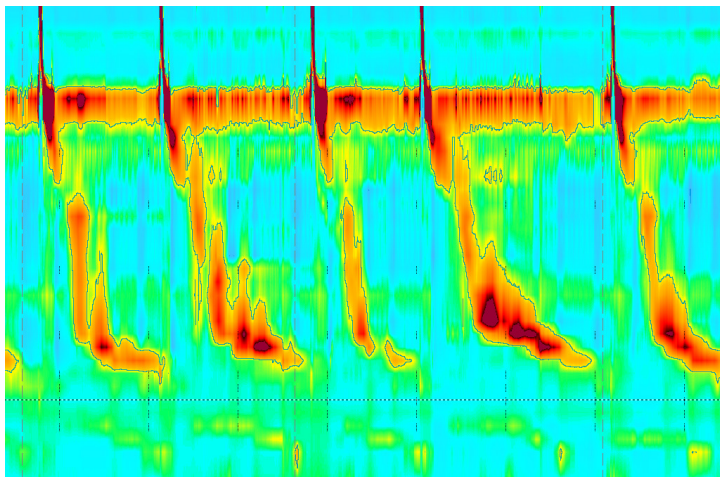
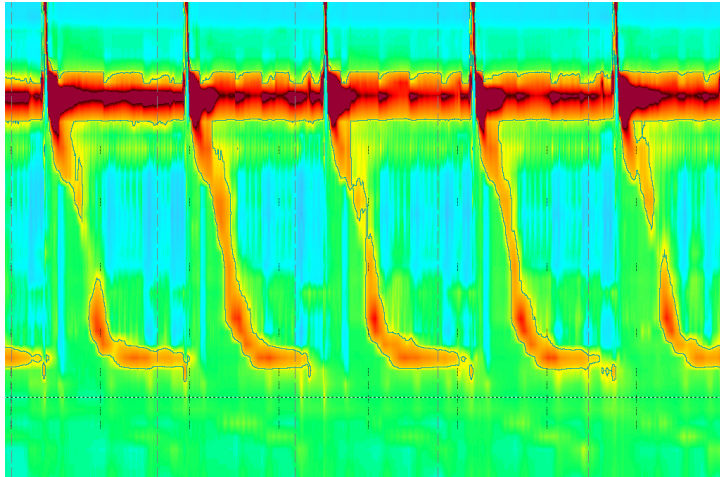


Figure 16 - Example of bread swallows compared with water swallow in the same patient: UP< swallows of water Down: bread swallows. (Image from internal source)

11.3.3 Oesophageal outlet obstruction with abdominal compression:

This test is used on both patients with IOM and/or GORD. Abdominal compression increases intragastric pressure provoking outlet obstruction to oesophageal bolus transport. In response, the normal oesophagus produces contractions of increased amplitude and duration in order to overcome the increased distal pressure and keep a normal bolus transit. Compared with the

baseline, the amplitude of oesophageal contractions increases with outlet obstruction in healthy subjects (29-30). However this does not occur in patients with irreversible hypomotility. These patients may not be able to compensate for outlet obstruction.

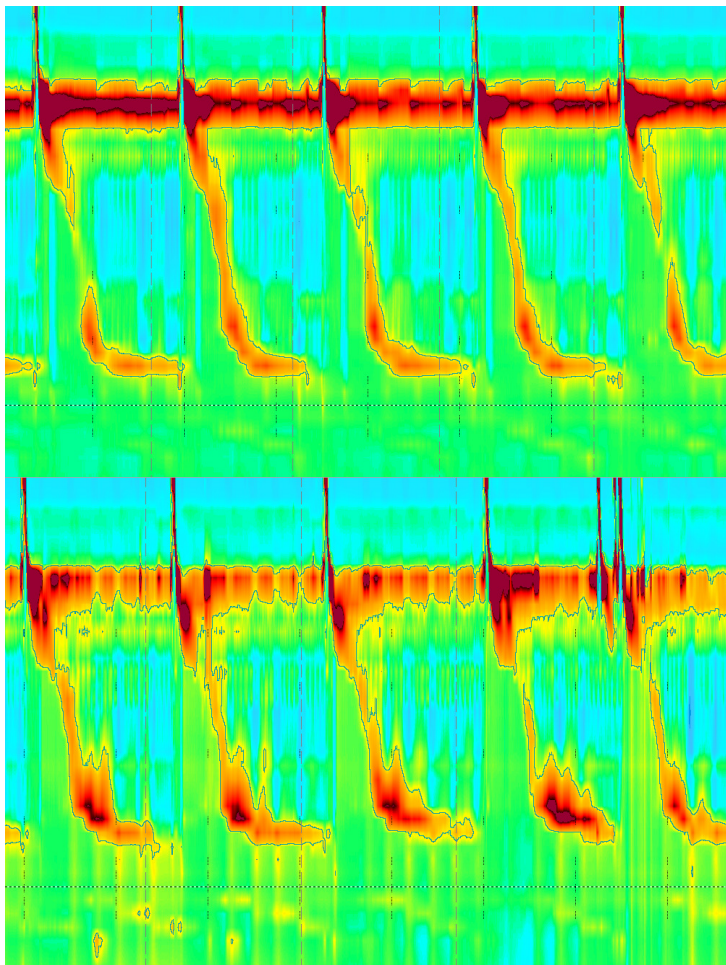


Figure 17 - Example of swallow with abdominal compression compared with water swallows in the same patient: UP< water swallow, Down: swallow of water with abdominal compression. (Image from internal source)

11.4 Physiology studies to investigate gastro-oesophageal reflux

11.4.1 24hour impedance-pH (reflux monitoring) - Technique

Even though many clinicians and investigators consider oesophageal pH monitoring the "gold standard" for measuring gastroesophageal reflux, this method has some inherent limitations. Impedance monitoring is the only recording method that can achieve high sensitivity for the detection of all types of reflux(235). The combination of both techniques is better than pH-metry or impedance monitoring alone (235). Impedance monitoring is based on measuring the resistance to alternating current (i.e., impedance) of the content of the oesophageal lumen. When a pair of electrodes, separated by an isolator (i.e., catheter) is placed inside the oesophagus, the electrical circuit is closed by electrical charges (i.e., ions) present in the oesophageal mucosa that surround the catheter. The 2.1-mm diameter combined MII-pH catheter is passed transnasally into the oesophagus and stomach and positioned so that the oesophageal pH electrode is located 5 cm above the proximal border of the LOS.

11.4.1.1 Reflux monitoring - Protocol

All patients underwent 24 h MII-pH monitoring. Patients had to be fasting from the night before and had to be off medication (any kind of PPI, prokinetics, opioid derived drugs, Erythromycin) for at least 5 days. A dedicated MII-pH catheter (with intraluminal impedance segments positioned at 3, 5, 7, 9, 15 and 17 cm above the LOS) (Sandhill Scientific Inc., Highlands Ranch, CO, USA) was placed transnasally, with the oesophageal pH sensor positioned 5 cm above the

manometrically determined LOS. Patients were requested to mark any symptoms that occurred along the recording time as well as every meal and change of position (to upright or recumbent) on the device. The catheter transmitted information into the software that was included in the device (Sleuth System – Sandhill Scientific Inc., Highlands Ranch, CO, USA). MII-pH data were collected and analyzed with the Bioview Analysis Software (Sandhill Scientific Inc., Highlands Ranch, Colorado, USA). By means of MII-pH it was determined whether the patient had pathological GOR or not (Figure 17). The cut off value of distal oesophageal acid exposure as percentage (%) of time with $\text{pH} < 4$ was considered abnormal if total time with $\text{pH} < 4$ was greater than 4.2%, and/or upright time with $\text{pH} < 4$ was greater than 6.3%, and/or recumbent time with $\text{pH} < 4$ was greater than 1.2%).

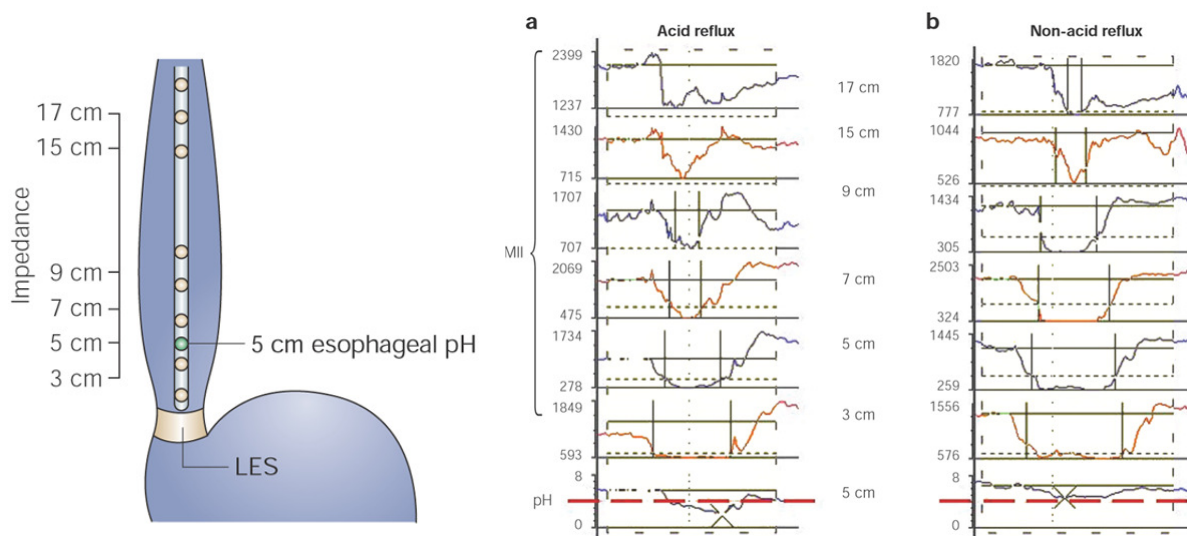


Figure 18 - Gastroesophageal reflux detected by combined multichannel intraluminal impedance and pH (MII-pH) monitoring. Impedance-detected reflux episodes during which the intraoesophageal pH drops from above to below 4.0 are considered acid (a), whereas impedance-detected reflux episodes during which the intraoesophageal pH remains above 4.0 are considered non-acid (b). From the following article: Gastroesophageal reflux monitoring: pH and impedance, Radu Tutuian and Donald O. Castell, GI Motility online (2006)

11.5 Physiology studies to investigate gastric emptying

11.5.1 Gastric emptying test: Technique

Breath tests have recently been developed and validated to allow the non-invasive and non-radioactive (hence used in this study to reduce the risks imposed on participants) measurement of gastric emptying which has shown significant correlation with the gold standard scintigraphic method (Hauser et al 2006). ^{13}C breath tests involve the measurement of the $^{13}\text{C}:^{12}\text{C}$ ratio present in breath carbon dioxide after ingestion of a nutrient meal, or other substrate containing ^{13}C . ^{13}C is a non-radioactive stable isotope that occurs naturally at an abundance of 1.1%. When a substrate that is relatively ^{13}C rich is ingested, the ^{13}C contained within it, after digestion and absorption, enters oxidative metabolic pathways. The end product of oxidative metabolism is $^{13}\text{CO}_2$, which is expired in the breath. This is separated from other components of expired air and its ^{13}C enrichment can be measured by infrared isotope ratio spectrometry. Breath samples are obtained before and at 30min intervals (15min interval for first 2hrs) after administration of the substrate for 4 hours. (Figures 18 and 19).

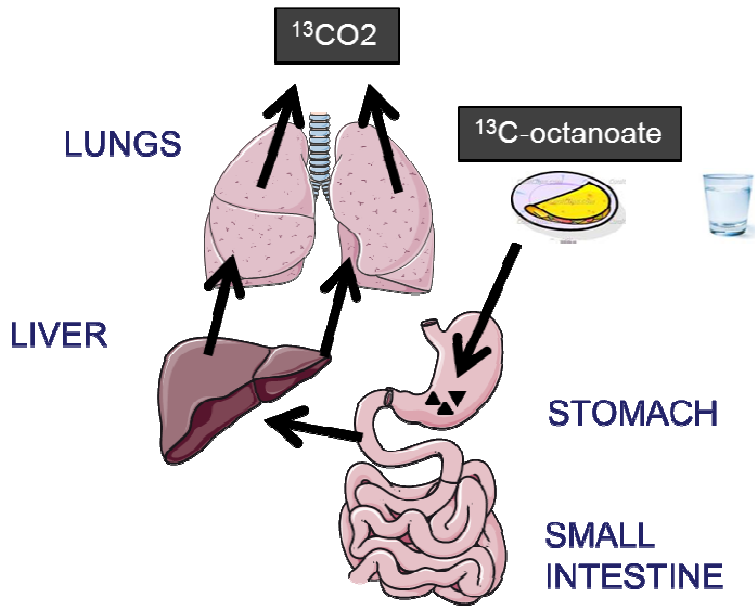


Figure 19 – Gastric emptying breath test with C^{13} -octanoic acid test meal.
 (Image source *Wagner Analysen Technik*)

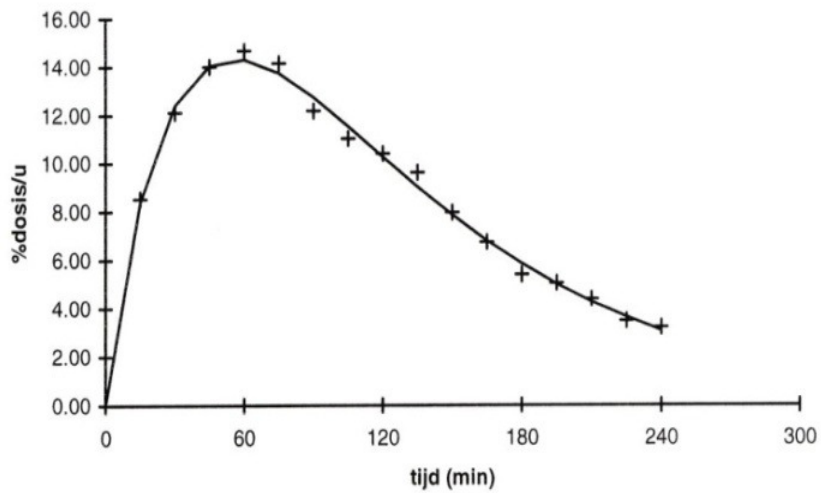


Figure 20 – Gastric emptying breath test analysis (a normal study curve).

11.5.2 Gastric emptying test: Protocol

Overnight fasted patients ingested a test meal of egg sandwich within 10 min. The meal comprised a scrambled egg with the yolk mixed with 100 mg of [^{13}C]octanoic acid and yolk and egg white cooked separately and two slices of white bread followed by 75 mL of still water. During the following 4-h test period the subjects stayed in a sitting position. Breath samples for $^{13}\text{CO}_2$ measurement were collected in breath bags every 15 min during the first 2 hours and thereafter in 30-min intervals. Control (baseline) sample was collected before the test meal.

Measurement of $^{13}\text{CO}_2$ in exhaled air was carried out by IRIS with the infrared spectrometer at our disposal (from Wagner Analystechnik, Worpswede, Germany).

11.6 Statistics

11.6.1 *General statistical considerations*

Statistical analysis for descriptive data such as means, prevalence, 5% and 95% were performed using column statistics by Prism software version 5 (Graph Pad, La Jolla, CA, USA). 2×2 contingency table, Student's t-test or chi-squared analysis as appropriate were applied to determine the significance level, considering $P < 0.05$ as statistically significant.

In chapter 3-2 we show the reproducibility of the Distal Contractile Integral (DCI). In statistical analysis of reproducibility, percentage coefficient of variation ($100 \cdot SD/\text{mean}$: %COV) was derived as a measure of inter and intraindividual variation. Moreover, intra-class correlation coefficient (ICC) and concordance correlation coefficient (CCC) were measured as other means of assessment of reproducibility. Bland–Altman plots were used to express the concordance of variables graphically.

In chapter 6, studying the effect of azithromycin on ineffective oesophageal motility, the following statistical considerations were applied. The primary outcome measure used in the analysis of the data to decide the effect of azithromycin or placebo is the distal contractile integral (DCI). The cut off level of DCI to diagnose IOM (236) is 447 ± 279.4 mmHg.cm.s. I expected that the increase of DCI post azithromycin therapy to be at least 50% above the baseline. For the calculation of sample size I took the level of significance of the test to be 0.05

and the power of the test to be 80%. The required number of participants in each arm (azithromycin and placebo) was calculated to be 13 subjects.

Comparisons between the Azithromycin and placebo group were made using contingency tables, and t test. *P*-values <0.05 were accepted as statistically significant. The data were presented as mean (\pm s.d.) or median (\pm interquartile range) as appropriate.

Finally, in studies of predictive values to identify responders to AZI the sensitivity, specificity and likelihood ratios were calculated based on the ROC curve to validate the most accurate predictive parameters.

11.6.2 Clinical trial of AZI vs Placebo on IOM - Primary Endpoint Efficacy Analysis

Increase of distal contractile integral (DCI) in the oesophageal body induced by AZI or placebo.

11.6.3 Secondary Endpoint Efficacy Analysis

1. Evaluation of changes in symptoms pre and post AZI therapy
2. Evaluation of manometric oesophageal body response to stimulation tests ie. solid bolus swallows, MRS and outlet obstruction in healthy subjects and patients with

IOM.

3. Effect of AZI on gastric emptying and gastroesophageal reflux

11.7 Research ethics committee approval

Studies on both healthy volunteer group and patient participants were approved by the NRES Committee: South East Coast – Kent and Sussex. Ethics committee reference number: 12/LO/0835 Queen Mary, University of London reference number: ReDA008188

CHAPTER 3-1

HIGH RESOLUTION

MANOMETRY:

NORMAL VALUES FOR HRM

USED IN THIS PHD COURSE

CHAPTER 3-1: HIGH RESOLUTION MANOMETRY: NORMAL VALUES FOR HRM USED IN THIS PHD COURSE

12 INTRODUCTION

Oesophageal motility studies are indicated for patients with dysphagia and non-cardiac chest pain, and may be useful in evaluating patients with gastro oesophageal reflux disease (GORD). Oesophageal motility was traditionally assessed by manometry employing a low compliance water-perfused catheter system. High resolution manometry with oesophageal pressure topography (HRM), overcomes several limitations of the conventional manometry by utilizing enhanced spatial pressure resolution and data visualisation. As a consequence, HRM widened the horizons for the understanding of oesophageal physiology and improvement in the clinical evaluation of oesophageal motor disorders (237). In addition, HRM is easy to perform and easily learned by the clinician(238). A major advantage gained in the adoption of HRM over conventional manometry has been the establishment of objective quantitative measurements of both oesophageal body motor response and GOJ relaxation (239-61).

Specific criteria for the interpretation of HRM and a new classification of oesophageal motility disorders (Chicago classification) were developed and have been improved (242), (59). Published normal values of HRM-specific metrics have been obtained from the Given Imaging HRM system, and it is not certain that these normative values necessarily apply to data derived

from other manufacturers' devices (244). This is a particularly important issue for Integrated Relaxation Pressure (IRP), a key measurement in the HRM criteria for achalasia diagnosis (239),(59). Although conceptually sound as a metric of GOJ relaxation, IRP is a technology-sensitive measurement, so that normal values presumably are specific for specific sensor types and arrays and the normative values of the metric must be linked to the assembly with which they were derived (239). Other oesophageal metrics are essential for the purpose of our research i.e. peristaltic break (PB) and distal contractile integral (DCI).

In the present PhD project an HRM assembly from Unisensor AG was used. To date, although more than 100 GI physiology research or clinical centres worldwide are using this system, normative values for HRM metrics were not established. Previous studies either used a different version of the HRM assembly (245) (a 36-channel solid-state unidirectional manometric catheter - Unisensor AG) or were performed on a particular population group (246). I established normative ranges and cut-off values for the HRM metrics derived from the Unisensor AG's 32channel solid state circumferential combined manometric catheter.

13 MATERIALS AND METHODS

13.1 Participants

Sixty-nine healthy volunteers (31 male, 38 female, mean age 30.33, age range 19-67) were included in this study. In this study, 35 volunteers were recruited in the UK and 34 volunteers recruited in Brazil. These healthy volunteers were clinically asymptomatic from both gastrointestinal symptoms as well as other significant medical conditions. None of the

participants were on regular medication except for oral contraceptive pills for some of the female participants. Ethics approval for running this study was obtained from the South East Coast – Kent. Ethics committee as well as the local ethics authorities in Brazil for the Brazilian participants and all the participants voluntarily signed informed consent before any assessment were performed for this study.

13.2 Oesophageal HRM

After overnight fasting, a 32channel solid state circumferential manometric monitoring catheter (Unisensor AG) was inserted transnasally at 60 cm from the nares. The participant lay down on their right lateral side and rested for 5 minutes before any measurements taken. The gastric baseline was recorded and the catheter was then repositioned in a way that both the upper and lower oesophageal sphincters could be identified simultaneously on the screen. The catheter was securely fastened to the face of the patient using adhesive tape. Basal oesophageal body and LOS measurements were recorded.

Using a syringe to inject water orally, a total of 10 single swallows of 5mls of water were performed with. at least 20 seconds interval between each swallow.

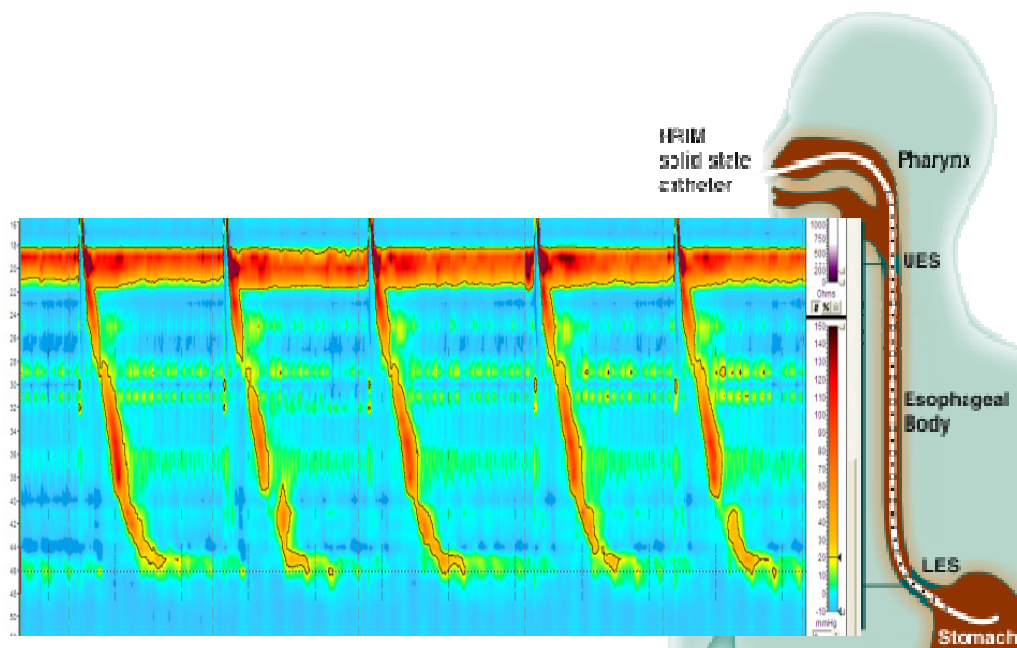


Figure 21 - Normal HRM tracing. High resolution manometry tracing and placement of catheter

13.3 Data analysis

Data was analysed using Bioview Analysis software version 5.6.3.0 (Sandhill Scientific Ltd.). The Chicago classification parameters (59) which are widely accepted and applied in the assessment of oesophageal high resolution manometry were used to characterize oesophageal motility pressure parameters. Thermal compensation was applied before any measurements were taken. Double swallows or swallows associated with belching or retching were excluded from analysis. The measurements of all the Chicago classification parameters as well as most of the generic parameters such as LOS and UOS pressure throughout this study required manual analysis. All measurements relating to LOS as well as intrabolus pressure were referenced to gastric baseline pressure. Oesophageal body contractility and UOS resting pressure were

referenced to atmospheric pressure. If after a swallow, no distal oesophageal contraction occurred, the parameters representing distal function could not be produced and subsequently these swallows were excluded from the analysis.

1 After manually positioning the required GOJ markers oesophageal contraction amplitudes at 5 and 10cm as well as the UOS resting pressure, the length of LOS and gastric pressure were measured using the analysis software.

2 All of the other parameters were measured manually including: LOS relaxation pressure and resting pressure, distal contractile integral (DCI), transition zone (TZ), contractile front velocity (CFV), Distal contractile latency (DL), Intrabolus pressure (IBP), Integrated relaxation pressures (IRP), contractile deceleration point (CDP). An isobaric contour of 20 mmHg was used throughout the analysis except for the measurement of transition zone for which isobaric contour of 40 mmHg was used.

3 The analysis software measured the amplitude of contractions automatically at 5 and 10 cm above GOJ.

4 GOJ upper and lower limits were marked at the highest and lowest borders recorded at end inspiration and end expiration respectively. LOS resting pressure was measured using a generic integrated tool in the analysis software at the end expiratory point referenced to intragastric pressure excluding the diaphragmatic crural effect. GOJ integrated relaxation pressures (IRP) 4-s was measured using a special tool integrated in the analysis software. GOJ relaxation was measured during a 10-s post deglutition time window in the electronically generated e-sleeve signal through the anatomic zone defined as the GOJ. GOJ nadir relaxation pressure was measured as the minimum pressure reached during 10 s post deglutition period.

5 The transition zone (TZ) was defined as the distance between the end of the proximal oesophageal segment and the beginning of the distal oesophageal segment in the 40-mmHg isobaric contour. The 20-mmHg isobaric contour used in other studies Smout (247) would mask this gap on most of the swallows. Brief peristaltic contractions shorter than 1 cm in length within the transition zone were ignored and were not considered as the beginning of the distal oesophageal segment.

6 The contractile front velocity (CFV) was defined as the slope of the line connecting the points on the 20-mmHg isobaric contour at the proximal and the distal margin of the distal oesophageal segment.

7 Distal contractile latency (DL) was defined as the interval between the start of UOS relaxation and the contractile deceleration point (CDP< the inflection point along the 20 mmHg isobaric contour where propagation velocity slows demarcating the tubular oesophagus from the phrenic ampulla).

8 The distal contractile integral (DCI) was calculated using integrated Chicago calculation tool in the analysis software which multiplies the length of the smooth muscle segment of the oesophagus generating the peristaltic contraction, by the duration of propagation of the contractile wave front and the mean pressure in the entire box excluding pressures below 20 mmHg.

9 Intrabolus pressure (IBP) was measured between the peristaltic wave front and the GOJ .

13.4 Statistical analysis and presentation of data

Data were tested for normality of the distribution using the Kolmogorov–Smirnov. Data are

presented as the mean, median and 5th and 95th percentiles. Normal values were defined as the interval between the 5th and 95th percentile of values.

14 RESULTS

Sixty-nine healthy volunteers successfully completed oesophageal high-resolution manometry with single swallows of water. Only values of distal latency and LOS resting pressure passed the Kolmogorof-Smirnof normality test.

14.1 Oesophageal peristaltic wave pressure topography

Table 1 provides manometric findings of oesophageal peristaltic parameters both from our study and in comparison with Chicago group (59) and the recent study from Smout et al (247). As shown in this table, the mean DCI was 1941 mmHg.s.cm, with a 5–95th percentile range of 606.7-4998 (median 1533 SD: 1492). The mean CFV was 3.95 cm/s, 5–95th percentile range 2-6.55. The mean DL was 6.94 s, 5–95th percentile range 5.19-8.81. The mean IBP was 9.93 mmHg, with a 5–95th percentile range of 1.97-17.61. The mean amplitude of contraction measured at 5 and 10 cm above the GOJ were 121.00 and 78.52.00 mmHg, respectively. The mean TZ length was 2.34 cm, 5–95th percentile range 0.00–6.00.

Table 1 - Comparison of the parameters in our study, study by Smout et al and the Chicago group.

	N	Mean	SD	Median	5 th	95 th	95 TH et. al	Smout	95 th Chicago
DCI	68	1941	1492	1533	606.7	4998	3407.60		<5000
IRP4	69	10.86	5.76	9	2.5	23.5	28.28		<15
CFV	68	3.95	1.28	4	2	6.55	6.50		<7.5
DL	68	6.94	1.11	6.9	5.19	8.81	8.70		<4.5
IBP	68	9.93	4.58	9.85	1.97	17.61	19		<15
AMP 5	68	121	61.15	110.5	43.8	260	146		<146.1
AMP 10	68	78.52	39.68	71	22.9	168.1	-		-

Figure 22 to Figure 28: Demonstrating the scatter plot for the Chicago parameters related to the oesophageal body measurements. TZ= transition zone, dci= distal contractal integral, cfv= contractile front velocity, DL= distal latency, IBP= intrabolus pressure.

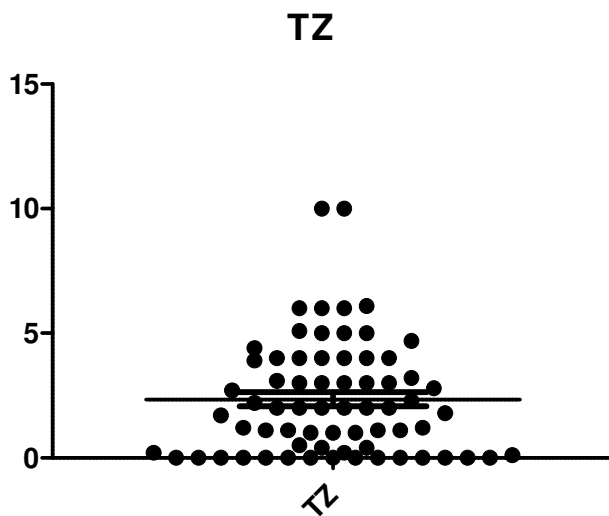


Figure 22 – (Figures 21-27: Demonstrating the scatter plot for the Chicago parameters related to the oesophageal body measurements.)

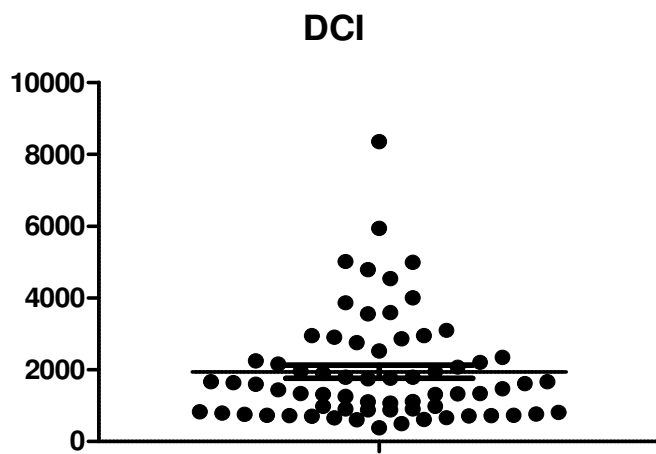


Figure 23

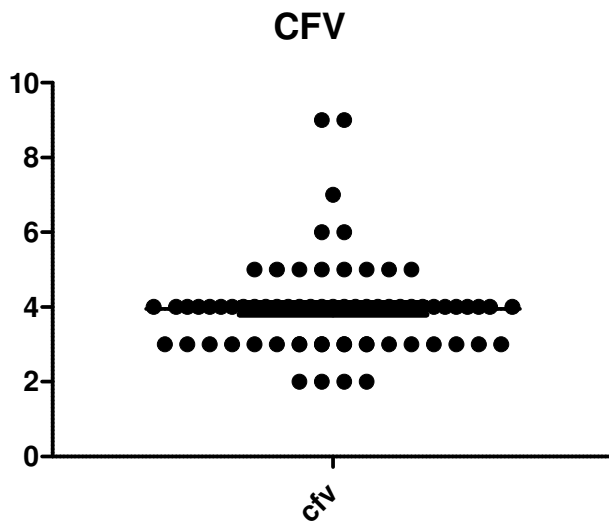


Figure 24

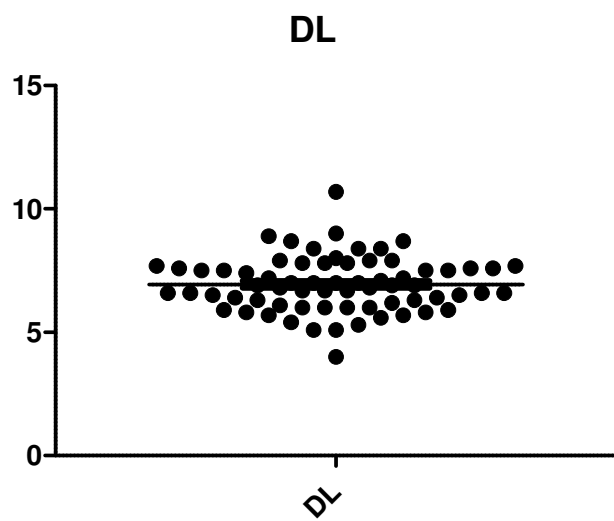


Figure 25

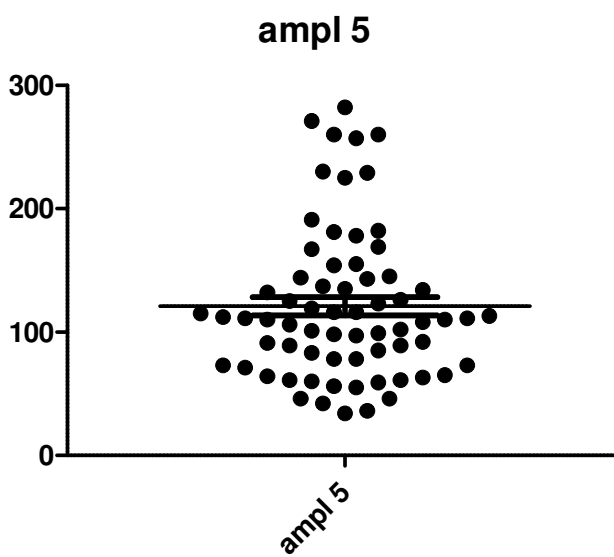


Figure 26

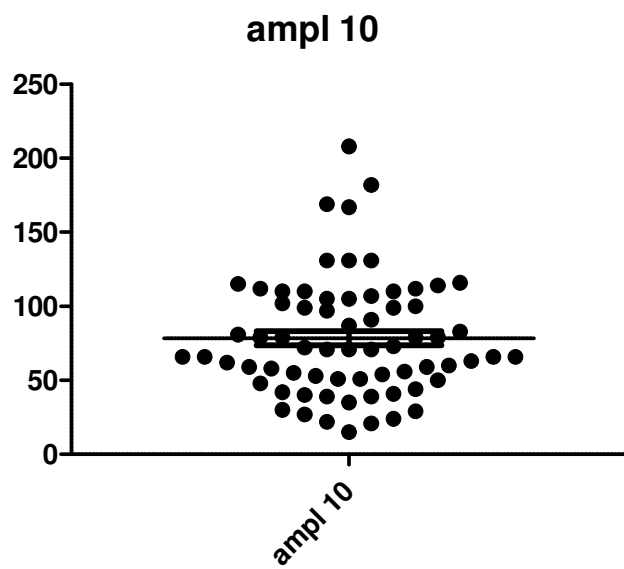


Figure 27

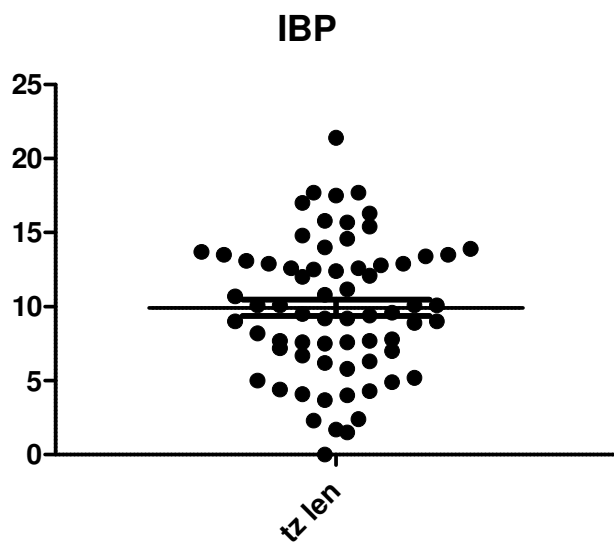


Figure 28

14.2 Sphincteric and gastric parameters

Mean UOS resting pressure was 78.84 mmHg with a 5–95th percentile range of 36.68-186.1 mmHg (Table 1). Mean GOJ length was 3.65 cm, with a 5–95th percentile range of 1.9-5.1 cm. Mean LOS resting pressure was 29.35 mmHg, with a 5–95th percentile range of 8.95–51.40 mmHg. Mean LOS nadir relaxation pressure was 4.9 mmHg, with a 5–95th percentile range of 0.10-10.45 mmHg. Mean IRP for 4 s was 10.86 mmHg, with a 5–95th percentile range of 2.5-6.55. Mean gastric pressure was 4.52 mmHg, with a 5–95th percentile range of 0.15-12.10 mmHg.

Figure 29 to Figure 35: Demonstrating the scatter plot and whisker and box plot for the Chicago parameters related to the sphincteric and gastric measurements. Vertical (y) axis corresponds to amplitude of the pressure mmHg.

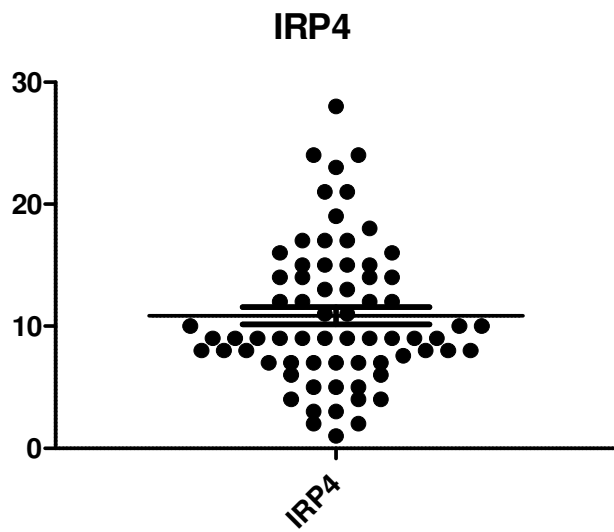


Figure 29 – (Figures 28-34: Demonstrating the scatter plot and whisker and box plot for the Chicago parameters related to the sphincteric and gastric measurements.)

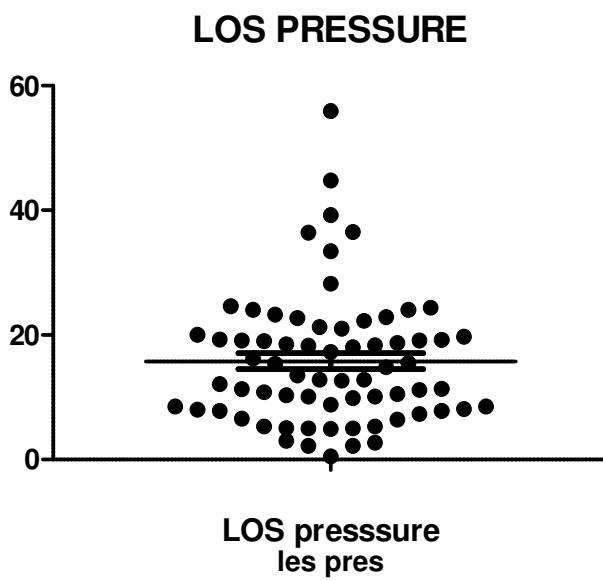


Figure 30

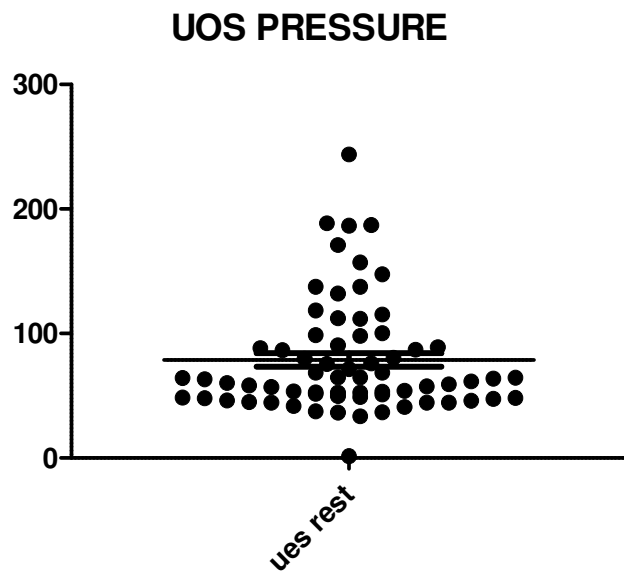


Figure 31

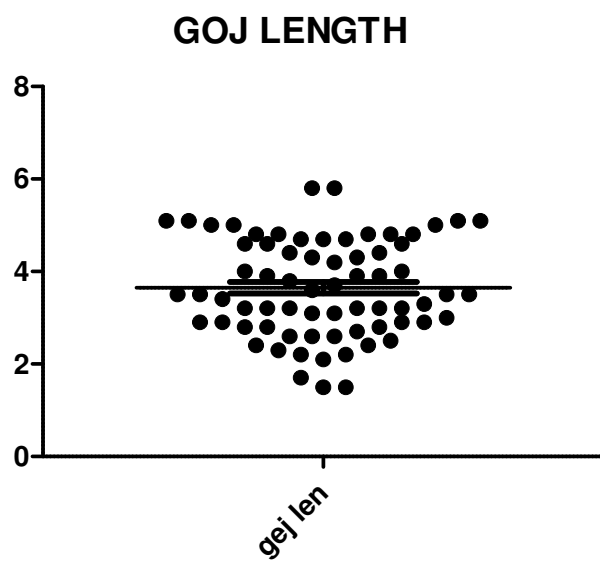


Figure 32

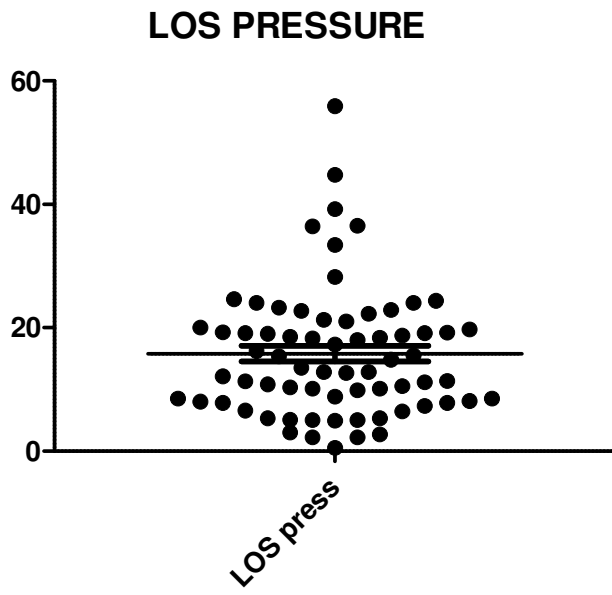


Figure 33

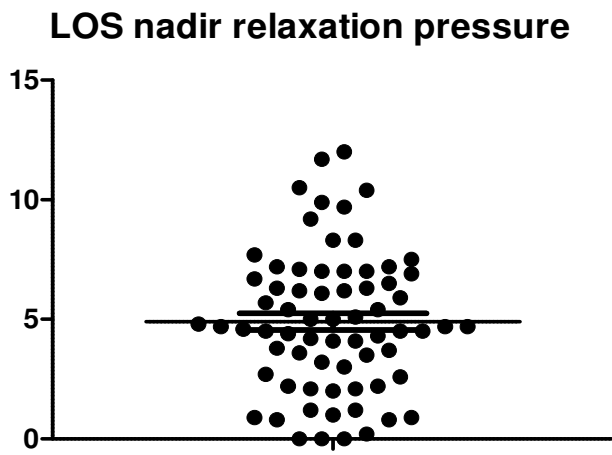


Figure 34

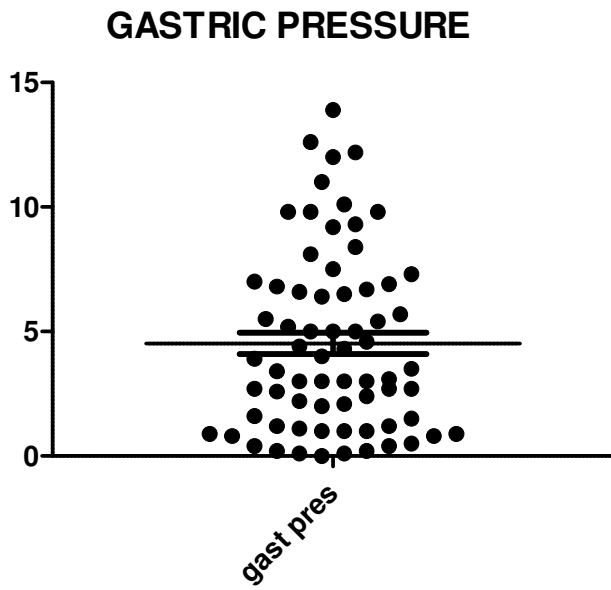


Figure 35

15 DISCUSSION

The evaluation of oesophageal motor function made by oesophageal manometry devices may be influenced by the manometric system employed. Therefore, it is recommended that normal values are established for each system. In the present chapter of this PhD thesis, the normative metrics for high-resolution manometry data obtained with the Unisensor AG assembly during water swallows in recumbent position in healthy volunteers are reported. The normal values of the parameters investigated in this study had some differences from those of the previous studies that used the MonoScan system. While the normal values for DCI in our study were similar to the data previously established by Chicago Classification, the CFV and DL cutoffs were slightly different. This fact may be the result of interobserver variability of measure of CFV and DL possibly due to the artifact produced by intra oesophageal pressurization (183). Even more important, the upper cutoff for IRP in the supine position utilized in the Chicago Classification of oesophageal motor disorders is 15 mmHg (248), whereas according to our results with the Unisensor AG HRM device it should be 23.5 mmHg. This is a particularly important issue because IRP is a key measurement in the HRM criteria to decide on the completeness of the LOS relaxation during diagnosis of achalasia (249). Our findings share similarities with those of Smout et al (247) who used Unisensor AG catheter, 3.3mm in diameter coupled to a MMS system (247); it is noteworthy that their IRP upper cut off (28 mmHg).

Our IRP results are also similar to those reported for a Chinese population by Shi Y et al, who used a Unisensor AG catheter with 4.2mm in diameter and a Sandhill system identical to ours (246). Since our study was conducted on volunteers recruited among Caucasian populations in UK and Brazil, this similarity suggests that racial differences do not influence GOJ function. One significant difference between our study and both the Chicago and Smout groups is the peristaltic amplitude at 5cm above LOS. This measure in our study yielded an amplitude nearly twice as of in the other studies. This result is partly due to the 4 outlier healthy participants with very high amplitude peristaltic contractions. Otherwise we could not explain the exact cause for this difference.

It is important to remember that this present study shows normative data for supine position. As it has been demonstrated by other studies, there are significant differences between HRM results in supine and upright positions, especially regarding the IRP and DCI (164, 250). Therefore, our normative results cannot be applied to the studies performed in sitting or upright positions.

15.1 Conclusion

Our results, taken together with those of Smout et al (247) and of Shi et al, indicate that HRM systems using the Unisensor AG catheter provides consistently higher IRP values in healthy volunteers than those of ManoScan system used to establish the Chicago classification. For the purpose of our work in this thesis, I used our normative values.

CHAPTER 3-2

Normal values and reproducibility of oesophageal stimulation tests

CHAPTER 3-2: Normal values and reproducibility of oesophageal stimulation tests

16 Introduction

In recent years, high-resolution manometry (HRM) has become the test of choice for the evaluation of oesophageal motor function and has been helpful in revealing previously unrecognized oesophageal physiologic mechanisms and pathophysiologic patterns. When oesophageal function is impaired due to ineffective oesophageal motility, due to the gradual loss of neuromuscular functionality, it is possible that there is still some functionality to be reserved. This reserved capacity of oesophageal contractility can be revealed in special challenging circumstances. Nevertheless, using single water swallows seems not to be sufficient to reveal the reserved oesophageal neuromuscular capacity. This is particularly important in making the distinction between reversible and irreversible oesophageal motor dysfunction which consequently can affect clinical decision making.

Patients presenting with oesophageal symptoms (dysphagia, chest pain, etc.) unexplained by endoscopy or barium studies are frequently referred for oesophageal high resolution manometry (HRM) to investigate a motor basis for symptoms (251). However, in many instances, the standard HRM may be normal in the presence of clinical symptoms or

while asymptomatic patients may have abnormal oesophageal motility. Consequently, provocative testing can give additional insight. Examples of provocative tests include multiple rapid swallows (MRS), solid bolus swallows (252), and abdominal compression test. Responses to provocative/stimulation tests may be useful in subtyping a spectrum of oesophageal disorders (253). Furthermore, these tests may predict oesophageal body contraction reserve and potentially assist in predicting the likelihood of postoperative dysphagia in patients undergoing antireflux surgery (254, 255).

16.1 Multiple rapid swallowing

MRS represents a simple provocative manoeuvre that can be easily incorporated into the oesophageal manometry protocol, and could demonstrate integrity of neural and motor processes in the smooth muscle oesophagus (256). Multiple swallows of water in rapid sequence induce central and peripheral neuronal inhibition of motor activity in the smooth muscle of the oesophagus and the lower oesophageal sphincter (LOS), and is dependent on intact inhibitory and excitatory neural function. A normal response during repetitive swallowing consists of inhibition of oesophageal body peristalsis and profound relaxation of the LOS (256, 257). After the last swallow of the series, there is a rebound excitatory response with an exaggerated oesophageal body peristaltic sequence and re-establishment of LOS tone following a brief after-contraction .

An abnormal response consists of either incomplete inhibition during the MRS, with contraction of the oesophageal smooth muscle during the expected inhibition phase, and/or suboptimal or absent peristaltic response after multiple swallows (256, 257). Multiple rapid swallows responses can be visually assessed during the inhibition and contraction phases on HRM . HRM quantitative parameters such as the distal contractile integral (DCI) can be used to quantitate the peristaltic response following MRS (255, 258). There are two versions of multiple repetitive swallows of water described in the literature, one with large volume of water i.e. 200ml (173) and another one with 10-15ml (256). It is believed that multiple swallow of large volume of water is more useful in the study of the retention of water i.e. in OGJ obstruction or achalasia rather than the study of oesophageal body motility. This is because large volume of water can at times distend oesophageal lumen to the extent that can prevent from initiating any contraction in the body. Multiple swallows of small amount of water is therefore more useful in identifying the potential vigor of oesophageal body in producing stronger response to the increased workload. Thus for the purpose of this PhD studies I employed the multiple swallows with small volume of water.

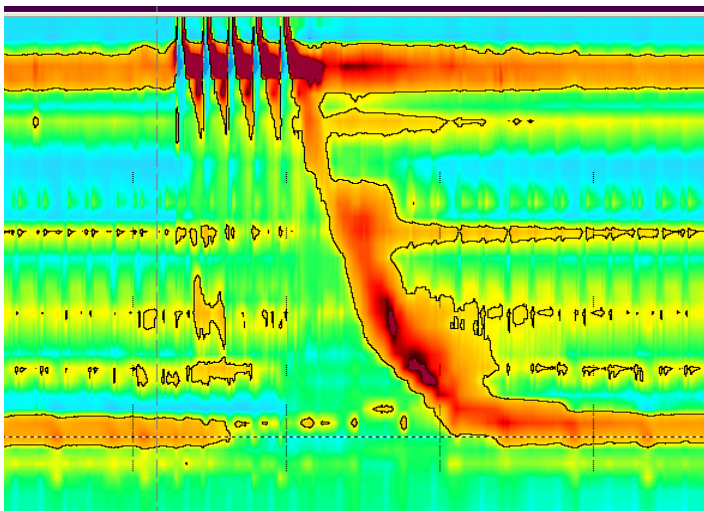


Figure 36. Example of a normal multiple rapid swallowing response.

16.2 Solid bolus swallow

In healthy volunteers, compared to water swallows, viscous or solid swallows show improved peristaltic co-ordination, increased contractile pressure and duration (259). An impaired response to solid swallows may be more clinically relevant than water swallows when the patient is tested in upright position. In this testing position the effect of gravity on transporting liquid bolus is more significant whilst solid bolus still requires some propulsive force from oesophageal body for transportation.

16.3 Abdominal compression

Increased abdominal pressure results in corresponding increase of intragastric pressure which in turn affects oesophageal peristaltic contraction and duration of peristalsis (260-264). This effect might be used as an additional test to assess the reserved capacity of the oesophageal muscle function. In animal models, increased intragastric pressure causes: 1) slowing of the peristaltic wave in the distal oesophagus, 2) increased pressure wave duration in the distal oesophagus, 3) increased oesophageal diameter, and 4) increased duration of lower oesophageal sphincter opening (263).

16.4 Normal values of provocative tests

Normal values for stimulation test are limited. Furthermore, similarly to normal values for standard liquid swallows HRM protocols, there is a need to establish normal values

for stimulation tests in each motility laboratory using specific HRM systems. With regards to the solid bolus swallows, Sweis et al, provided normal values using a HRM Given system (164).

In this chapter I provide quantitative normal values for all stimulation tests using our Unisensor HRM catheter and Sandhill system. I also assessed the reproducibility of the three stimulation tests – multiple rapid swallows (MRS), abdominal compression test and bread swallows to address the interindividual variability of these manometric findings.

17 METHODS

17.1.1 Participants

The study population consisted of 26 healthy subjects median age of 23.5 years, ranging from 19-52 years. Seventeen were female (54%).

recruited to undergo high-resolution manometry in the Upper GI Physiology Unit at the Royal London Hospital. All subjects were required to have no significant foregut symptoms and not taking any form of medication that might affect gastrointestinal motility in any way and/or gastric secretion. Those with previous surgical intervention of the upper gastrointestinal tract were also excluded. Each of the volunteers underwent oesophageal HRM by solid-state catheter, Unisensor AG assembly with 32 sensors, in two separate sessions at least one week apart.

Studies on healthy volunteers was approved by the NRES Committee: South East Coast – Kent Ethics committee reference number: 12/LO/0835 Queen Mary, University of London reference number: ReDA008188

17.1.2 High resolution manometry

HRM studies were conducted using an Unisensor AG assembly (Figure 36). The High resolution manometry probe consisted of 32 pressure, 12 Fr, each with 12 circumferential pressure sensors. All studies were analyzed using the most recent software version Bioview analysis software available from Sandhill Scientific at the time of the analysis (version 5.6.3.0).



Figure 37 - Unisensor AG high resolution assembly.

Prior to the test, subjects were fasted for at least 6 hours. Topical anesthetic was applied into the nostril, followed by transnasal intubation of the oesophagus. Catheter placement included initially at 60cm from nares in order to record the gastric baseline and then the catheter was repositioned in order to position one or two pressure channels above the upper oesophageal sphincter (UOS), and 2-3 cm below the crural diaphragm (CD). Deep inspiration helped to identify the CD (increased pressure amplitude of the CD, as well as increased intra-abdominal and decreased intra thoracic pressure).

Subjects were studied in the semi-recumbent position. Subjects were allowed 5–10 minutes to accommodate to the presence of the catheter without coughing or choking. This was followed by ten 5 mL water swallows, separated by 20 seconds of interval. The OGJ and oesophageal body were allowed to return to their resting state prior to each of the 10 swallows.

17.1.3 Stimulation tests

- 1) Multiple rapid swallowing (MRS): After baseline HRM recording of 10 single swallows of 5 ml water, three sets of MRS - 2ml water every 1-2 seconds, 5 times was performed.
- 2) Abdominal compression test: Next after multiple swallows of water, an inflatable waist belt was fitted around the subject's waist. The pressure cuff was inflated and the inflation of the cuff continued until a pressure of between 100-180 mmHg was

achieved on external pressure manometry gauge depending on the tolerance of the patient. The subject was offered 10 swallows of 5ml water at not less than 30 seconds intervals (**Error! Reference source not found.**).

- 3) Solid bolus swallows: After allowing 5-10 minutes rest to the subject, ten bread swallows (pieces 2-3 cm³)(164) of white bread without crust were conducted. The subject was allowed to chew the bread freely until ready to swallow in one single swallow.

17.1.4 Assessment of the gastro-oesophageal junction and oesophageal body

GOJ relaxation is studied using integrated relaxation pressure (IRP). IRP is taken relative to intragastric pressure. To determine the IRP, using a computer tool first the upper and lower margins of the OGJ is determined, and then a 10-second time window is identified that begins at the start of LOS relaxation initiated by swallowing. This tool measures pressure simultaneously over the length of the rectangle drew over the 10 second distance. Then, it calculates maximum pressure along the height of the rectangle at each time point within the 10-second time window. The 4-second IRP algorithm takes these pressures and averages the lowest of them, the nadir pressure, over 4 continuous or discontinuous seconds. Using 4 discontinuous seconds to determining nadir pressure

eliminates cardiovascular artefacts, and pressures produced by contraction of the crural diaphragm during inspiration from calculation of the IRP.

Assessment of oesophageal body characteristics is defined based on the current parameters introduced by Chicago classification including measures of (i) breaks in the isobaric contour of 20 mmHg, (ii) circular muscle strength using contraction amplitude and distal contractile integral (DCI); and (iii) wave propagation using contractile front velocity, distal latency (CFV and DL). Each parameter is measured in the distal (smooth muscle) oesophageal body rather than the proximal (striated muscle). Pressure measurements of the oesophageal body are taken relative to atmospheric pressure.

Breaks in the integrity of peristalsis were assessed in the 20 mmHg isobaric contour (measured using the specific tool in the Bioview software). To determine peristaltic integrity, a 20 mmHg isobaric contour line is applied to the HRM. A threshold value of 20 mmHg above which intact oesophageal peristalsis is defined was chosen originally by the Chicago consensus group because this is the peristaltic pressure required for normal bolus transit when the OGJ is functioning normally. Peristaltic integrity is assessed by measuring gaps in the 20 mmHg contour along the length of the oesophagus, between the UOS and LOS.

Circular muscle contraction amplitudes is measured at 5cm level of the distal segment of the oesophageal body and expressed as global measure of distal contractions – the distal contractile integral (DCI). The software allows automated measure contraction amplitude

at the distal 5cm along the length of the oesophageal body. The peak of the pressure upstroke reflects the contraction amplitude and is taken relative to the oesophageal baseline pressure of that particular channel. I measured the contraction amplitude at 5cm above the proximal border of the OGJ as an additional indicator of the oesophageal body strength.

There are 2 measures to evaluate propagation of oesophageal pressure waves; the "contraction front velocity (CFV)" and the "distal latency (DL)". The CFV is a measure of peristaltic velocity in the smooth muscle oesophagus; that is, from the distal extent of the transition zone to a landmark called the "contractile deceleration point (CDP)" (265). The CDP is the time point during a peristaltic pressure wave at which peristalsis in the distal oesophagus appears to slow appreciably. Functionally the CDP is the time at which oesophageal peristalsis terminates, and the LOS begins to descend to its resting position. Descent of the LOS is seen radiographically as emptying of the phrenic ampulla. Wave propagation is defined as the CFV is expressed as cm/s. The CFV is obtained by calculating velocity from a best linear fit along the 20 mmHg isobaric contour line at the leading edge of the peristaltic pressure wave from transition zone to CDP. The CFV can appear rapid when the bolus is pressurized between an unyielding OGJ and a peristaltic contraction. This situation might be mistaken by automated analysis software as a simultaneous contraction. This circumstance can be remedied by choosing an isobaric contour pressure that exceeds pressure at the GOJ.

The distal latency is not a measure of peristaltic contraction velocity. Instead, it identifies the time from the start of swallow induced UOS opening to arrival of oesophageal contraction at the CDP (266). It is presumed to measure post deglutitive inhibition and adequacy of inhibitory neuromuscular function in the smooth muscle oesophagus (267, 268). A short DL indicates early arrival of the oesophageal contraction in the distal oesophagus. It is now used instead of rapid CFV in the Chicago classification to diagnose distal oesophageal spasm because it more reliably identifies patients with this disorder (269).

The DCI reflects the calculation of integrated pressures above 20 mmHg from the upper border of the LOS to the lower border of the transition zone and is used to measure the robustness of peristaltic contraction in the smooth muscle oesophagus. The DCI integrates pressure, distance and time along the oesophagus (269). The analysis is performed by making a box that encompasses all swallow induced motor activity produced by contractile segments S2 and S3. The DCI is calculated by summing pressures > 20 mmHg from all of the time/length foci within the box. It is basically an aggregate of the mean contraction amplitude of the smooth muscle oesophagus, the length over which that contraction propagates, and duration of contraction. All efforts are used to avoid including intrabolus pressure or pressure produced by vascular structures in the calculation of DCI.

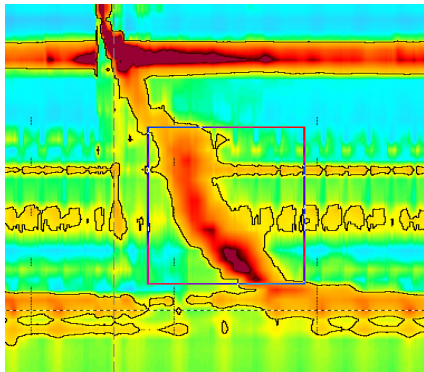


Figure 38 - Area of peristaltic contraction used for calculation of DCI (in the square). Source of image, internal.

17.1.5 Data analysis

To assess the effectiveness of the stimulation tests in provoking stronger response in the oesophageal body, the DCI achieved by each stimulation test is compared against the DCI of the single swallows of 5ml water. According to the Chicago Consensus, DCI is considered as the indicator of the oesophageal contractility (59).

Normal values for all the major parameters used in the Chicago Classification were established in each of the stimulation tests. These include: integrated relaxation period (IRP), distal contractile integral (DCI), peristaltic break (PB), distal latency (DL) and contractile front velocity (CFV) (59). Variables are expressed as the mean plus or minus standard deviation or as median with range. Fifth and 95th percentile values were calculated and taken as lower and upper limits of the normal variations.

The main variable to quantify the peristaltic contractility in studying the reproducibility of the stimulation tests was Distal Contractile Integral (DCI). In statistical analysis of reproducibility, percentage coefficient of variation ($100 \cdot SD/\text{mean}$: %COV) was derived as a measure of inter and intraindividual variation. Moreover, intra-class correlation coefficient (ICC) and concordance correlation coefficient (CCC) were measured as other means of assessment of reproducibility. Bland–Altman plots were used to express the concordance of variables graphically.

18 RESULTS

In evaluating the effectiveness of the oesophageal stimulation tests in provoking more vigorous contraction in oesophageal body, DCI was compared between stimulation tests and single swallows of 5ml water (Figure 39 and Figure 40). As demonstrated in Table 2, the P values of the change of DCI in stimulation tests versus baseline swallows of 5ml water were statistically significant. Table 3 demonstrates the comparative ranges for the stimulation tests versus routine single swallows of water.

Table 2 - Effectiveness of the stimulation tests: this table demonstrates that how different is DCI comparing water swallows and each of the stimulation tests. P values confirm that there is a significant change of DCI by implying each of the stimulation tests.

	Effectiveness	Effectiveness	Effectiveness
Effectiveness of the stimulation tests	MRS DCI	PRESS DCI	BREAD DCI
Comparative values	vs	vs	vs
(Paired t test)	DCI WATER	DCI WATER	DCI WATER
P value	0.0001	< 0.0001	0.0002
Mean of differences	934	627.8	668.2

Table 3 - Comparative ranges for the stimulation tests versus routine swallows of water

Effectiveness of the stimulation tests	DCI WATER	DCI PRESS	DCI MRS	DCI BREAD
Descriptive values				
Number of values	26	26	26	26
25% Percentile	716.8	1291	1349	1361
Median	1208	2017	1800	1771
75% Percentile	1904	2407	2769	2133
5.000% Percentile	421.0	844.8	732.2	739.2
95.00% Percentile	3861	4221	6741	4402
Mean	1375	2003	2309	2043
Std. Deviation	893.8	888.0	1566	1032

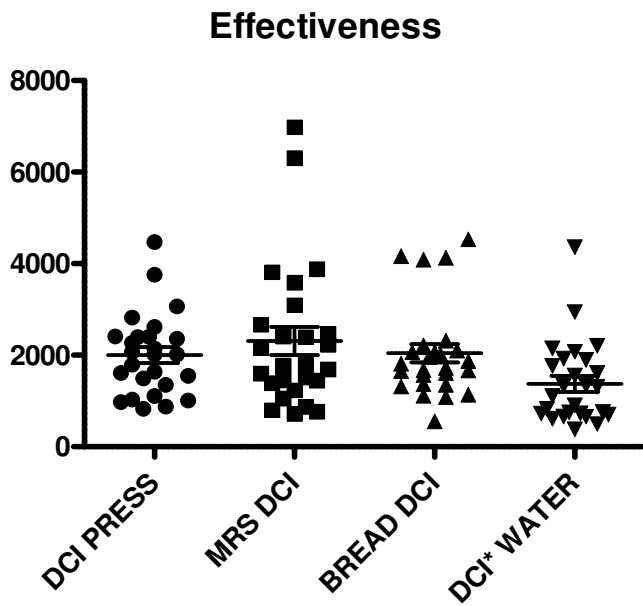


Figure 39 - Comparison of the stimulation tests versus routine swallows of water.

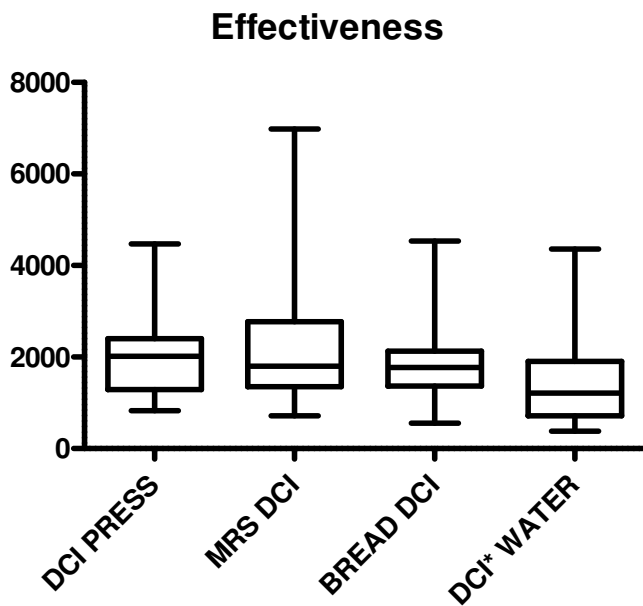


Figure 40 - Comparison of the stimulation tests versus routine swallows of water.

MRS measurements –The normal values are defined with 3 sets of MRS.

Normal mean residual pressure (4 s-IRP) was 5.5 mmHg (95th percentile: 12.3 mmHg) .

Contraction amplitude of oesophageal circular muscle. was 134.4 mmHg (95th percentile: 216 mmHg, 5th percentile: 68 mmHg).

The mean DCI was 2160 mmHg cm s, median DCI 1727 mmHg second cm (95th percentile: 6741 mmHg second cm, 5th percentile: 250 mmHg cm s) with a maximum of 6977 mmHg cm s.

Abdominal compression measurements -The normal values are defined with swallows of 5ml water with average increase in intragastric pressure of 11.8 mmHg, with a range of 3.6 – 26.4 mmHg, median = 11.6 mmHg. Gastric baseline pressure with abdominal compression was used as the reference to calculate the IRP.

Normal mean residual pressure (4 s-IRP) was 7.6 mmHg, range of 0-16 mmHg (95th percentile: 16 mmHg).

Contraction amplitude of oesophageal circular muscle was measured at 5 cm above the OGJ. At five centimetres above the OGJ, the mean contraction amplitude was 129.6 mmHg (95th percentile: 195mmHg, 5th percentile:72 mmHg).

The mean DCI was 2003 mmHg cm s, median DCI 2017 mmHg second cm (95th percentile: 4221 mmHg second cm, 5th percentile: 844.8 mmHg cm s) with a maximum of 4471 mmHg cm s.

Bread swallow measurements – The normal values for bread swallows are defined using 10 swallows of 3 cm³ of bread (Table 6). Normal mean residual pressure (4 s-IRP) was 10.7 mmHg, range of 3-20 mmHg (95th percentile: 19.6 mmHg).

Contraction amplitude of oesophageal circular muscle was measured at 5 cm above the OGJ. At five centimetres above the OGJ, the mean contraction amplitude was 137.3 mmHg (95th percentile: 278.7 mmHg, 5th percentile: 69.2 mmHg).

The mean DCI was 2043 mmHg cm s, median DCI 1771 mmHg second cm (95th percentile: 4402 mmHg second cm, 5th percentile: 739 mmHg cm s) with a maximum of 4531 mmHg cm s.

Tables 4-6 demonstrate manometric normal values for each stimulation test. Table 7 provides normal values for single swallows of 5ml water in order to facilitate the comparison with stimulation tests. Tables 8-13 demonstrate comparison of different parameters between stimulation tests and water swallows.

Table 4 - Normal values for MRS

	DCI	CFV	DL	PB	IRP	Ampl 5cm
Number of values	26	26	26	26	26	23
Median	1800	4	6.55	0.15	5.5	129.0
5.000% Percentile	732.2	3	4.84	0	-1.3	68.00
95.00% Percentile	6741	6.3	11.31	3.13	12.3	216.0
Mean	2309	3.962	6.885	0.7192	5.423	134.4
Std. Deviation	1566	0.9992	1.605	1.017	3.744	46.16

Table 5 - Normal values for abdominal compression test

	DCI	CFV	DL	PB	IRP	Ampl
Number of values	26	26	26	26	26	25
Median	2017	3	6.65	1	7.9	128.0
5.000% Percentile	844.8	2.35	4.675	0	-0.95	72.00
95.00% Percentile	4221	5.65	8.96	3	16	195.3
Mean	2003	3.577	6.75	0.8654	7.608	129.6
Std. Deviation	888.0	0.8566	1.203	0.9753	4.8	33.11

Table 6 - Normal values for bread swallows

	DCI	CFV	DL	PB	IRP	Ampl
Number of values	26	26	26	26	26	25
Median	1771	3.5	7	0	10.5	127.0
5.000% Percentile	739.2	2	5.535	0	3	69.20
95.00% Percentile	4402	8.6	11.97	3.37	19.65	278.7
Mean	2043	3.769	7.554	0.4808	10.77	137.3
Std. Deviation	1032	1.608	1.643	0.9051	5.331	49.10

Table 7 - Normal values for single swallows of water

	DCI	CFV	DL	IRP4	PB	AMPL
Number of values	26	26	26	26	26	25
Median	1027	4.000	7.300	8.000	3.000	98.50
5.000% Percentile	467.8	2.000	4.825	1.750	0.0	40.00
95.00% Percentile	3096	5.000	8.750	14.25	10.00	236.5
Mean	1330	3.647	7.194	7.559	3.326	102.0
Std. Deviation	778.3	0.8121	0.9692	3.501	2.535	51.52

Comparative normal values for stimulation tests:

Table 8 - Comparative normal values for stimulation tests DCI

Normal values	DCI of MRS	DCI of Pressure	DCI of bread	DCI of water
Number of values	26	26	26	34
Median	1800	2017	1771	1027
5.000% Percentile	732.2	844.8	739.2	467.8
95.00% Percentile	6741	4221	4402	3096
Mean	2309	2003	2043	1330
Std. Deviation	1566	888.0	1032	778.3

Table 9 - Comparative normal values for stimulation tests PB

Normal values	PB of MRS	PB of Pressure	PB of bread	PB of water
Number of values	26	26	26	34
Median	0.15	1	0	3.000
5.000% Percentile	0	0	0	0.0
95.00% Percentile	3.13	3	3.37	10.00
Mean	0.7192	0.8654	0.4808	3.326
Std. Deviation	1.017	0.9753	0.9051	2.535

Table 10 - Comparative normal values for stimulation tests CFV

Normal values	CFV of MRS	CFV of Pressure	CFV of bread	CFV of water
Number of values	26	26	26	34
Median	4	3	3.5	4.000
5.000% Percentile	3	2.35	2	2.000
95.00% Percentile	6.3	5.65	8.6	5.000
Mean	3.962	3.577	3.769	3.647
Std. Deviation	0.9992	0.8566	1.608	0.8121

Table 11 - Comparative normal values for stimulation tests DL

	DL bread	DL pressure	DL MRS	DL water
Number of values	26	26	26	34
Median	7	6.65	6.55	7.300
5.000% Percentile	5.535	4.675	4.84	4.825
95.00% Percentile	11.97	8.96	11.31	8.750
Mean	7.554	6.75	6.885	7.194
Std. Deviation	1.643	1.203	1.605	0.9692

Table 12 - Comparative normal values for stimulation tests Amplitude at 5cm above LOS

Normal values	Amplitude MRS	Amplitude bread	Amplitude Pressure	Amplitude water
Number of values	23	25	25	34
Median	129.0	127.0	128.0	98.50
5.000% Percentile	68.00	69.20	72.00	40.00
95.00% Percentile	216.0	278.7	195.3	236.5
Mean	134.4	137.3	129.6	102.0
Std. Deviation	46.16	49.10	33.11	51.52

Table 13 - Comparative normal values for stimulation tests IRP

Normal values	IRP of MRS	IRP of Pressure	IRP of bread	IRP4 of water
Number of values	26	26	26	34
Median	5.5	7.9	10.5	8.000
5.000% Percentile	-1.3	-0.95	3	1.750
95.00% Percentile	12.3	16	19.65	14.25
Mean	5.423	7.608	10.77	7.559
Std. Deviation	3.744	4.8	5.331	3.501

To assess the reproducibility of the stimulation tests, DCI is compared in visit one and visit two (Figure 41 to Figure 43). The choice of DCI is due to the fact that this multifactorial parameter is the key parameter in the evaluation of IOM used in this PhD thesis. HRM showed significant change of DCI during all stimulation tests compared to the single water swallows (P value < 0.05 in all stimulation tests). There was no significant difference for DCI values between visit one compared to visit two in each stimulation test (MRS P value = 0.8380, pressure P value = 0.4112, bread swallows P value = 0.5637). This means that the DCI figures in visit one are reproduced in visit two. As additional tests of reproducibility, two more assessments were conducted. Coefficient of variation analysis which showed minimal differences between inter and intra-individual %COV indicating reproducibility of the stimulation tests. Significant but not perfect concordance values were found for all stimulation tests (CCC bread = 0.77, CCC MRS = 0.74, CCC pressure belt = 0.64). ICC showed high values for intra-individual reproducibility for all stimulation tests, the highest being for bread swallows (ICC average measures = 0.87). Figure 44 to Figure 46 show the Bland–Altman plots for DCI in MRS, abdominal compression test and bread swallows. In these plots the data points are relatively closely scattered around the x-axis, indicative of a small difference between the two measurements as compared to the mean of the two measurements.(Table 14)

Table 14 - Bland–Altman parameters for reproducibility

Bland–Altman parameters for reproducibility	MRS	ABDOMINAL COMPRESSION TEST	BREAD SWALLOWS
Bias	-43.56	-122.8	-87.27
SD of bias	1075	749.2	760.4
95% Limits of Agreement			
From	-2150	-1591	-1578
To	2063	1346	1403

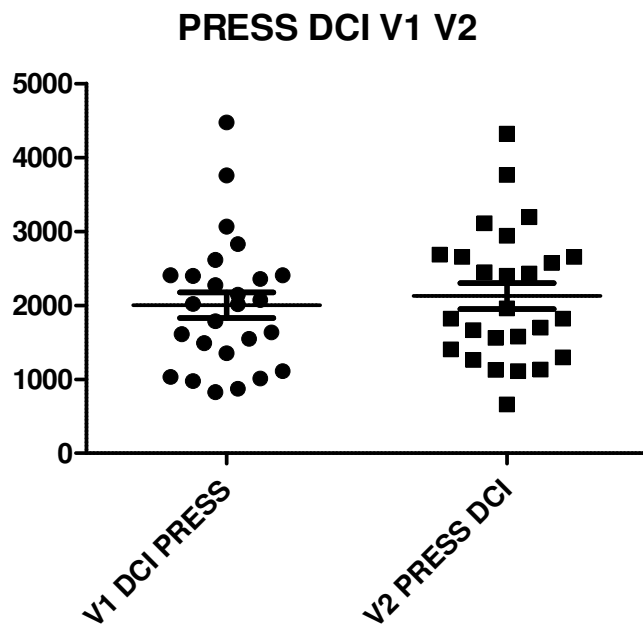


Figure 41 - Comparing DCI of vисти 1 and 2 for abdominal compression test

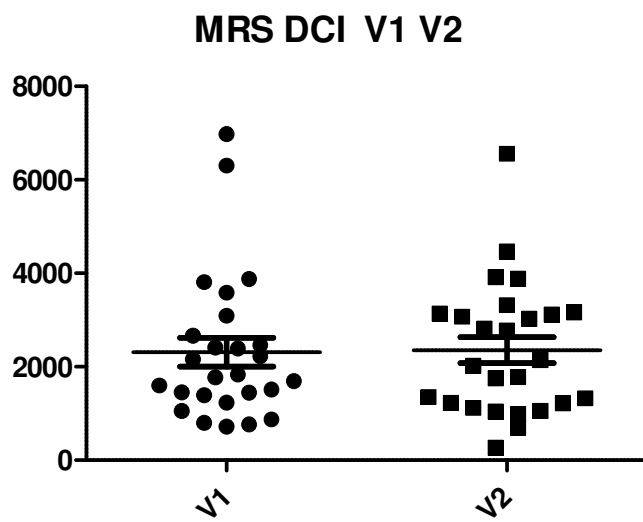


Figure 42 - comparing DCI of vисти 1 and 2 for MRS

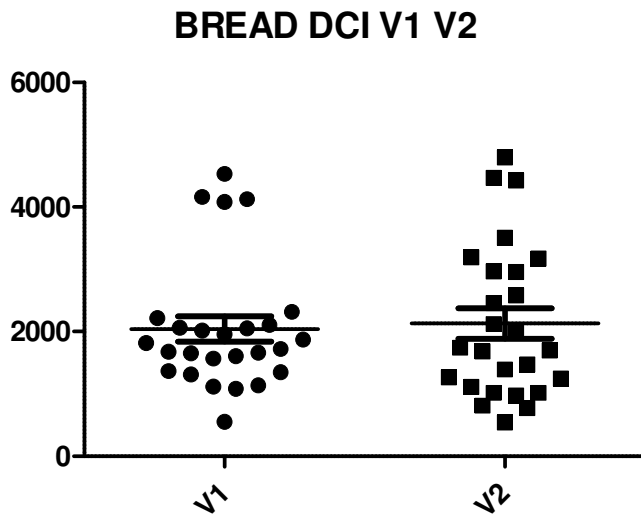


Figure 43 - Comparing DCI of visit 1 and 2 for bread swallows

Bland-Altman of PRESS DCI V1 V2: Difference vs average

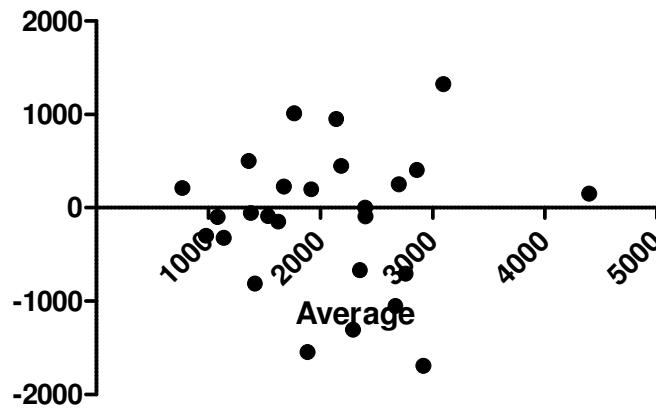


Figure 44

Bland-Altman of MRS DCI V1 V2:Difference vs average

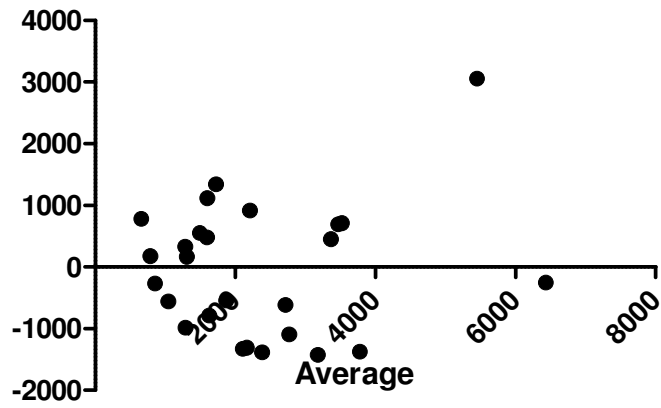


Figure 45

Bland-Altman of BREAD DCI V1 V2:Difference vs average

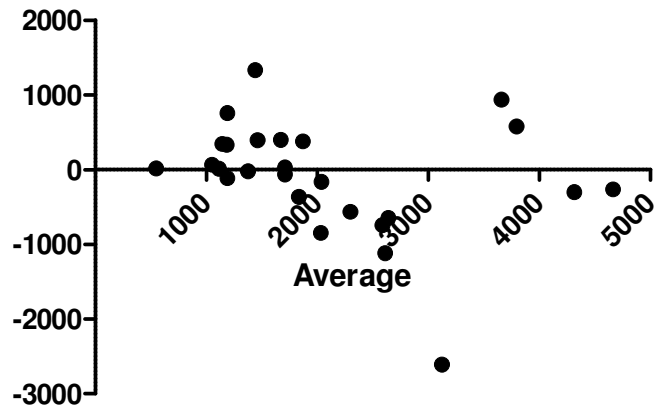


Figure 46

19 Discussion

The most appropriate and clinically relevant measurement protocol for oesophageal stimulation tests has not been established. Current diagnostic classifications for conventional and HRM are based on repeated small volume water swallows in the supine position (55, 248). Some authors have recommended performing stimulation tests such as solid swallows (164), to increase sensitivity to symptomatic dysmotility and dysfunction; however the pressure record is more complex under these conditions and reference values have not been established. This study presents normative values for HRM parameters of peristaltic and OGJ function that predict effective liquid and solid bolus transport (159, 171, 64, 272) in multiple rapid swallowing, bread swallows and swallows with abdominal compression. These data provide a systematic analysis of ‘normal’ high-resolution manometry thresholds in 26 healthy volunteers using Unisensor AG HRM assembly. The data include metrics of the recently published Chicago classification.

19.1.1 Effect of stimulation test on oesophageal contractility in healthy subjects

Assessment of the effect of the oesophageal stimulation tests on the oesophageal body contractility is defined based on the distal contractile integral (DCI)(255). The distal contractile integral (DCI) is an index of contractile vigor in high-resolution oesophageal pressure topography calculated as the product of amplitude, duration, and span of the distal oesophageal contraction (59). The mean of the differences in DCI produced by

each of the stimulation tests were compared against single water swallows. All three stimulation tests were able to induce DCIs significantly higher than single swallows of water. The mean of the difference of DCI for bread swallows and abdominal compression were 668.2 mmHg and 627.8 mmHg respectively (P values = 0.0001). MRS achieved the highest mean of the DCI differences amongst the stimulation tests, mean of the difference = 934 mmHg, P value = 0.0001

19.1.2 Effect of stimulation test on OGJ relaxation in healthy subjects

GOJ relaxation is studied using integrated relaxation pressure (IRP). The summary of the mean IRP (mmHg) finding is as following (IRP during abdominal compression is not discussed here because due to the nature of this test, the results are technically inappropriate):

MRS (5.4) < Water (7.5) < Bread (10.7)

MRS yielded in the lowest IRP amongst all the swallowing tests. The reason for such a low IRP with MRS, which is even lower than single water swallows, is most likely due to the prolonged inhibition of LOS during multiple swallows of water. Multiple swallows provide enough time for the LOS to relax completely and there is no increased intrabolus pressure as in bread swallows. From previous studies (164, 256, 273) it was expected that

there would be an increase in IRP for solids compared to liquids because this parameter increases not only with LOS dysfunction (i.e. impaired relaxation and opening), but also with increased friction between the bolus and the luminal wall (61). Our study confirms this concept in which the difference of IRP mean is the highest in bread swallows.

Assessment of oesophageal body characteristics is defined based on the current parameters introduced by Chicago classification including measures of (i) breaks in the isobaric contour of 20 mmHg, (ii) circular muscle strength; contraction amplitude and DCI; and (iii) wave propagation; CFV and DL.

Effects of bolus consistency and load on oesophageal function were consistent with previous studies using conventional and high-resolution manometry (159, 274, 275). Overall, as expected from previous studies (164), as workload increased, oesophageal contractile response was slower [lower contraction front velocity (CFV)], better coordinated (shorter PTZ) and more vigorous [greater distal contractile integral (DCI)] This comparison is clear for solid versus single swallows of water but it is hard to decide which stimulation test bears higher workload compared to the other. In table 15 comparison of the findings in regards to CFV, PB and DCI is demonstrated.

Table 15 - comparison of the findings in regards to CFV, PB and DCI

CFV mean cm/s	Pressure(3.5)	Water (3.6)	Bread (3.7)	MRS (3.9)
PB mean cm	Bread (0.4)	MRS (0.7)	Pressure (0.8)	Water (3.3)
DCI,mean,mmHg.cm.s	Water (1330)	Bread (2043)	Pressure(2066)	MRS (2309)

It is clear that peristaltic break i.e. transition zone during all three stimulation tests were significantly lower than in single swallows of water (mean of the PB in MRS, abdominal compression, bread and water swallows = 0.7, 0.8, 0.4 and 3.3 cm). PB in bread swallows was the lowest amongst all different types of swallows. This finding makes bread swallows the best stimulation test amongst others to test the continuity of the peristaltic wave as well as the coordination of striated-smooth muscle in transition zone.

Mean DCI, a global measure of distal oesophageal circular muscle strength, ranged from 2003 mmHg in swallows with abdominal compression to 2309 mmHg in MRS. Bread swallows stands in between these two stimulation tests with 2043 mmHg. Therefore, MRS seems to be the stimulation test with the highest provocative capacity to induce stronger contractility in oesophageal wall. 95 percentile of the DCI achieved by MRS is beyond the 5000 mmHg limit of the normal DCI defined by the Chicago group (95% DCI of MRS = 6741 mmHg).

I calculated peak amplitude at 5cm above the proximal border of the OGJ as reference value. Our data clearly shows an increase in average peak contraction amplitude achieved by stimulation tests compared to single swallows of water in the distal oesophagus. Bread swallows induced the highest contraction amplitude at 5cm level followed by MRS and abdominal compression: 137.3 mmHg, 134.4 mmHg, 129.6 mmHg respectively.

The mean CFV from bread swallows and MRS were higher than water swallows measuring in descending order: MRS (3.9 cm/s) > bread swallows (3.7 cm/s) > pressure 3.5 (cm/s).

As expected the DL of the bread swallows was higher than water swallows (mean = 7.5 sec). Surprisingly the DL of the abdominal compression and MRS were both shorter than single swallows of water (mean DL of MRS and compression 6.8 sec and 6.7 sec respectively). This finding indicates that two of the stimulation tests (abdominal compression and MRS) induce faster peristaltic contraction. Comparing the DL and CFV of the stimulation tests should yield in similar ranking however the finding is that although abdominal compression induces the shortest DL, it has the highest CFV.

DL: bread > MRS > pressure

CFV: MRS > bread > pressure

19.1.3 Assessment of the reproducibility of the stimulation tests

In this study, overall reproducibility of stimulation tests in oesophageal HRM data was good and this can be considered as an important validation of the reliability of these new techniques.

As was shown, there was minimal difference between inter and intra-individual %COV (table 16). In addition, concordance testing showed that significant but not perfect concordance values were found for all stimulation tests. Although considerable absolute variations occurred between the first and the second measurement, the values stayed within the normal range in these healthy subjects, limiting the importance of these variations. Most importantly, in the first and the second measurements, no large differences were found for DCI.

Table 16 - COEFFICIENT OF VARIATIONS

COEFFICIENT OF VARIATIONS			
	VISIT 1 %COV	VISIT 2 %COV	TOTAL %COV
	Intra-individual variation of DCI	Intra-individual variation of DCI	Inter-individual variation of DCI
BREAD SWALLOWS	50.5	57.6	54.0
MRS	67.8	60.3	63.5
ABDOMINAL COMPRESSION	44.3	42.0	42.8

It should be realized that even large variations in DCI between different days are only of importance when they change the overall conclusion of the measurement. Whether the observed day-to-day changes in the measured parameters will affect overall conclusion of

the measurement can only be answered in a study in which patients are measured twice (276). Our concordance data showed that at least in healthy volunteers, the day-to-day variability does not frequently change values in a way that they would alter the final conclusion of the test. This supports the use of stimulation tests as additional tools in the clinical evaluation of the patients with oesophageal motor disorders in order to assess the reserve oesophageal neuromuscular capacity.

CHAPTER 4

Oesophageal stimulation tests and symptoms in patients with IOM

CHAPTER 4: Oesophageal stimulation tests and symptoms in patients with IOM

20 INTRODUCTION

Ineffective oesophageal motility (IOM) is characterized by a weak oesophageal motility response to swallows associated with poor bolus transit in the distal oesophagus. Ineffective motility occurs when 30-50% (according to Spechler and Blonoki) (55) (277) or more of swallows are followed by contraction amplitudes of less than 30 mmHg at 3 or 8 cm above the lower oesophageal sphincter (55) (277). Ineffective oesophageal motility is believed to be an important pathologic feature of both gastroesophageal reflux disease (GORD)(278) and non-obstructive dysphagia (68) (279).

With the advent of high-resolution manometry (HRM) and oesophageal pressure topography, there are new metrics to define oesophageal motor function. Measures of peristaltic integrity and vigour involve both peristaltic amplitude and breaks in the peristaltic wave front. The new metric is called distal contractile integral (DCI). In the Chicago Classification of oesophageal motility, the definition of weak peristalsis is based on the length of breaks in the 20 mmHg isobaric contour (IBC), as these have been shown to be associated with impaired bolus transit with both fluoroscopy (280) and intraluminal impedance monitoring(60). Also important in the description of weak

peristalsis is the location of these breaks, as they may have distinct pathologic origins and consequences. Xiao et al utilized DCI to define ineffective swallows(236). Their data suggest that the manometric correlate of IOM in HRM is a mixture of failed swallows and IBC break in the middle/ distal troughs. A DCI value<450 mmHg-s-cm can be utilized to predict ineffective oesophageal swallow. IOM can be defined by >50% swallows with weak /failed peristalsis or with a DCI <450 mmHg-s-cm.

In spite of new and better definitions of IOM, its clinical relevance is not completely clear. Furthermore, in some patients IOM is associated with dysphagia whereas in other IOM patients underlies poor oesophageal clearance of gastro-oesophageal reflux.

The stimulation tests used in this study. – (multiple swallows of water, increasing outlet resistance at GOJ by applying abdominal compression, bread swallows) - theoretically use different pathways to stimulate oesophageal contraction. Multiple rapid swallows particularly requires intact deglutitive inhibition and preserved excitatory mechanism to provoke after MRS contraction (281). Bread swallows requires preserved afferent pathway to detect the presence of the solid bolus in the oesophageal body and effective excitatory efferent pathway to enhance the strength and coordination of oesophageal contractions (282). Abdominal compression requires an intact mechanism that detects increased resistance at the GOJ level and increases excitatory pathways to augment the strength of contraction to overcome the resistance and assure oesophageal emptying (234, 256, 263, 283).

I hypothesised that 1) IOM is associated with a specific defective inhibitory or excitatory mechanisms that regulates oesophageal body motility. 2) This failure can be specific for

different symptoms and 3) Such specific mechanism failure can be predicted by different oesophageal stimulation tests

The aim of this study was to assess the relationship between response to oesophageal stimulation tests and symptoms profile (dysphagia, heartburn/regurgitation, cough) in patients with IOM.

21 Methods

21.1 Subjects and study protocol

Patients referred for oesophageal high resolution manometry who were diagnosed with IOM according to the modified Chicago Classification (236) were selected. Patients should: 1) present with either one or a combination of: reflux symptoms (heartburn and/or regurgitation) and dysphagia. Patients were excluded if: 1) motility disorders other than IOM such as spastic contractions or OGJ obstructions existed, 2) Barrett's oesophagus larger than 3 cm (endoscopic evidence), 3) hiatus hernial larger than 3cm.

21.2 High resolution manometry protocol

Please see chapter 2 for the details of high resolution manometry protocol.

21.3 Reflux monitoring protocol

Please see chapter 2 for details of reflux monitoring protocol.

To assess the symptoms, all patients were provided with a symptom questionnaire to highlight their main symptom.

21.4 HRM analysis

The HRM plot of each swallow was analyzed for integrity of the 20 mmHg isobaric contour. Peristalsis was defined as intact if no break longer than 2 cm was observed in the IBC. Failed peristalsis was defined by minimal (<3cm) integrity of the 20mmHg isobaric contour distal to the proximal pressure trough. When the 20mm Hg isobaric contour was disrupted, the length of the break was measured using the dedicated tools in each of the analysis software. Weak contractions were categorized as weak contraction with large breaks (>5cm in length) or weak contraction with small breaks (2–5cm in length). The Distal Contractile Integral (DCI) was calculated as the mean amplitude (greater than 20 mmHg) of the distal oesophageal contraction in mmHg-s-cm(59). The final diagnosis of the HRM for every patient was made according to the 2012 version of the Chicago Classification: ‘weak peristalsis with large peristaltic defects’ if greater than 20% of swallow exhibited large (>5 cm) breaks in the 20 mmHg IBC, ‘weak peristalsis with small peristaltic defects’ if greater than 30% of swallows exhibited small (2–5 cm) breaks in the 20 mmHg IBC, or ‘frequent failed peristalsis’ if >30% but <100% of swallows were associated with failed peristalsis(59). For the purpose of this study I excluded the patients diagnosed with ‘weak peristalsis with small peristaltic defects’. I defined severe ineffective oesophageal motility (IOM) as being either: ‘weak peristalsis with large peristaltic defects’ or ‘frequent failed peristalsis’. Absent peristalsis was not included in this study.

Changes in DCI were used to evaluate the response to stimulation tests. A normal response to a stimulation test was defined when the DCI changed between 5 and 95 percentiles observed in normal subjects. Using these criteria, normal response was defined as a DCI >732 mmHg.sec.cm for MRS, >844 mmHg.sec.cm for abdominal compression and >739 mmHg.sec.cm for bread swallows. (See following figure)

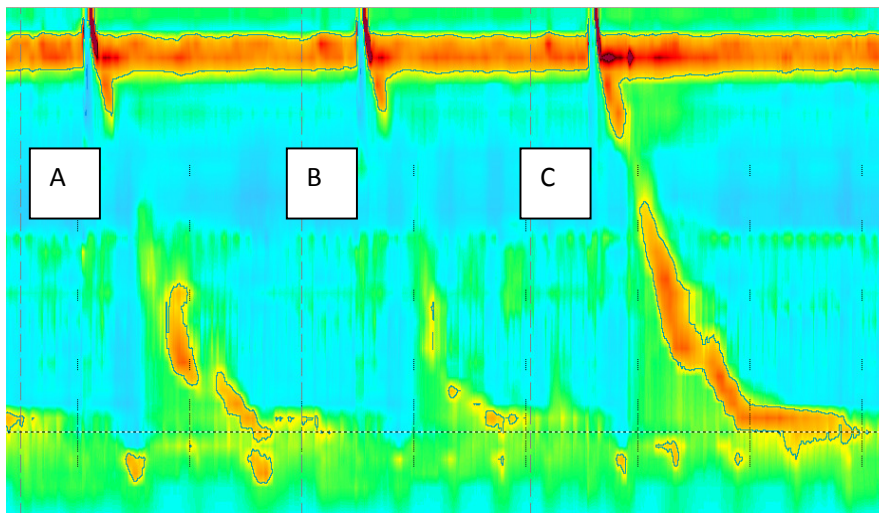


Figure 47 - Different types of peristalsis defects: A) weak peristalsis with large peristaltic defect, B) failed peristalsis, C) weak peristalsis with small peristaltic defect.

21.5 Statistical analysis

The results of DCIs were expressed as either normal or abnormal. For each patient I identify the predominant symptom as being dysphagia or reflux (heartburn/regurgitation) Combinations of the responses to stimulation tests were considered to further identify the most significant defective pathways in the oesophageal body motility system. Comparisons among these variables were made using contingency tables, and Fisher's exact test. A *P*-value <0.05 was accepted as statistically significant in all analyses.

22 Results

In total, 42 patients were included, 22 male and 20 female with the age range of 25-70 years old, median age 56 years old. There were 9 patients with hiatus hernia (<3cm) detected in total, 6 from reflux group and 3 from dysphagia group.

The symptoms included: 32 patients who predominantly presented with reflux symptoms either heartburn or regurgitation and 10 patients with dysphagia as their dominant symptom.

22.1 High-resolution manometry

7/42 patients had normal response to all stimulation tests (2/10 with dysphagia and 5/32 with reflux).

In the reflux group, the incidence of abnormal multiple rapid swallow was 16/29 (55%), abnormal abdominal compression 17/31(54.8%) and abnormal bread swallows 22/32(68.7%) [the reason for different total number of each test is that some participants did not manage to complete one or the other test]. In the dysphagia group the incidence of abnormal multiple rapid swallow was 4/10 (40%), abnormal abdominal compression 7/10 (70%) and abnormal bread swallows 5/10 (50%). The most common abnormal response to stimulation tests was seen for bread swallows with 28 out of 42 patients (5/10 in dysphagia group and 23/32 in reflux group).

All three stimulation tests were abnormal in 14 patients (33.3%). This was more common in the patients whose main symptom was reflux (13/32, 40.62%) compared to the patients with dysphagia (1/10, 10%) although the P value was not statistically significant (P value = 0.4229). 9/32 patients with reflux symptoms had hiatus hernia of which 4 (44.5%) patients had abnormal response to all stimulation tests and 2 had normal response to all. 15/32 patients with reflux symptoms were found to have pathological gastro-oesophageal reflux of which 6 (40%) had all responses abnormal and 2 all normal.

The IRP was normal in all patients. However, IRP was slightly higher in patients with dysphagia compared to patients to patients with reflux symptoms (median IRP 9.1 mmHg versus 6.7 mmHg, P value = 0.04).

23 Discussion

IOM is one of the most common oesophageal motility findings in GI physiology units (58% of all the diagnosis) (66). It is also the most prevalent oesophageal motor disorder in GORD, found in 21–38% of patients in large series (69, 71-73). Moreover, IOM was present in 27–32% of patients presenting with non-obstructive dysphagia without GORD (67-70). However, the role of IOM in pathophysiology of reflux symptoms and dysphagia is still a matter of debate. It is not clear why one motility pattern i.e. IOM can be associated with different symptoms profile (reflux dominant and dysphagia dominant). In this study I used three different stimulation tests to assess the neuromuscular integrity of the oesophageal body in patients with severe hypomotility. I hypothesized that a distinct response to stimulation test would predict the predominant symptom. However, our

findings did not support such hypothesis. There was no clear difference in response to stimulation tests between patients with predominant reflux symptoms compared to patients with predominant dysphagia.

More than one third of patients with reflux symptoms and pathological acid GOR(37,5%) had all three stimulation tests abnormal whilst this finding was the least frequent in the dysphagia group (10%).

Having hiatus hernia increased the likelihood of having abnormal response to all stimulation tests (45% of the patients with hiatus hernia had all responses abnormal).

Although the group differences between reflux and dysphagia patients was not significant, I observe a trend suggesting that reflux patients were more likely to fail response in all three stimulation tests. Based on this trend, I can just speculate that the mechanism underlying OH in reflux disease is different from that in dysphagia. Tutuian et al reported that a higher proportion of oesophageal motility abnormalities during bread swallows was observed in patients with chest pain and GORD symptoms compared to patients with dysphagia (284).

The IRP in patients with dysphagia was slightly higher than in the reflux group suggesting that increased intrabolus pressure associated with a higher distal resistance might be more relevant to dysphagia sensation than the severe hypomotility alone. In contrast, patients with lower IRP (reflux group) had more abnormal response to all three stimulation tests, suggesting that a better motility response to stimulation tests has less impact in dysphagia perception.

What are the potential mechanisms that trigger increased contractions during stimulation tests? Oesophageal mechano-receptors (stretch sensors) are probably initially implicated in the 3 stimulation tests, in the outlet obstruction induced by abdominal compression, the increased resistance against the passage of bolus and peristaltic propelling pressure provokes increased wall expansion and stretch. Such stimulus can trigger a peripheral reflex (in the oesophageal wall) resulting in increased circular and longitudinal smooth muscle contraction in the segment above the bolus via a cholinergic, muscarinic mechanism (285, 286). Furthermore, changes either in the preload (muscle length or stretch) or afterload (the mass of the bolus) induces contraction with higher amplitude (287, 288). Finally, pressure sensors in the abdominal cavity or stomach might stimulate the afferent limb of a vagovagal reflex arch modulating oesophageal peristalsis (287, 289).

Similar mechanisms can explain the effect of bread swallows. However, the after contraction at the end of multiple rapid swallowing probably requires an additional central input from CNS. During repetitive swallows, there are central inhibitory signals transmitted to the oesophageal body causing hyperpolarisation of the smooth muscle cells leading to a strong after-contraction (290).

In normal subjects, the different stimulation tests used in this study are able to trigger increased oesophageal contractility. They use peripheral and central pathways. The MRS uses more central and the abdominal compression and bread swallows more peripheral pathways. In my study I used the tests to assess reserve capacity of patients with severe

hypomotility. I identified a group of patients with appropriate reserve capacity (hypomotility in basal condition and increased contractility during stimulation).

23.1 Limitations

The lack of differences between groups could be due to Type 2 error due to small number of dysphagia patients.

I did not have simultaneous confirmation of the bolus retention during swallows that could be associated with dysphagia. However, for reflux symptoms, I could link these symptoms to pathological GOR objectively because it was possible to measure retention of refluxate corresponding to each symptom.

23.2 Summary

In conclusion, the present study used high-resolution manometry using multiple rapid swallowing, abdominal compression and bread swallows to identify differences between patients with IOM who present with dysphagia and GORD symptoms. I could not demonstrate significant differences in response to stimulation tests between reflux and dysphagia patients. However, I identified a trend towards a more severe failure (i.e. worse reserve capacity) in reflux patients compared to dysphagia.

CHAPTER 5

Proximal oesophageal

hypomotility:

definition, prevalence and

clinical relevance in

patients with severe distal

hypomotility

CHAPTER 5: Proximal oesophageal hypomotility: definition, prevalence and clinical relevance in patients with severe distal hypomotility

24 Introduction

While the Chicago classification has extensively characterized contractions of the distal smooth muscle oesophagus and the length of transitional zone, pathology of proximal (striated muscles) oesophageal motility is not included in this classification (291). However, there are patients with weak or absent proximal oesophageal contractions with normal or abnormal distal oesophageal motility. The clinical relevance of this finding is unknown. Previous studies with standard manometry have described abnormalities affecting only the striated muscle portion of the oesophagus such as myasthenia gravis(292) and polymyositis(293). So far, emphasis has been given to the lower oesophageal motility in the development of clinical symptoms and little attention has been paid to the role of proximal oesophageal motility in this regard.

The normal values for the proximal oesophageal motility were defined using a high resolution manometry system(291) (Sierra Scientific Instruments). A 10 mmHg isobaric contour was used to define the boundaries of the proximal oesophageal contraction area. In our studies, I use a different HRM device (Sandhill Scientific Inc., Highlands Ranch, CO, USA) and I used a 20 mmHg isobaric contour, which provides more precise discrimination between proximal and distal motility areas Figure 48.

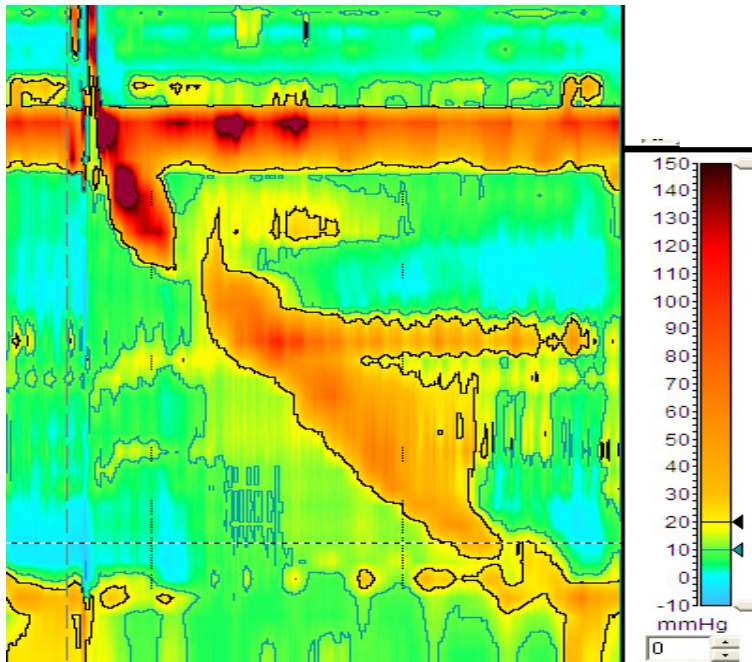


Figure 48 – The difference of applying 20 mmHg (black) versus 10 mmHg (grey) isobaric contour. When 10 mmHg isobaric contour is used, there is more chance that the noise surrounding the actual contraction area will be included in measurement of DCI. (Image from internal source)

.This study aims to (1) establish normative values for proximal oesophageal motility (2) assess the prevalence of proximal OH in the population of patients with severe distal OH (3) identify the clinical relevance of the proximal OH to symptoms.

25 Material and Methods

25.1 Subjects

25.1.1 *Healthy volunteers*

Manometric studies were performed on 30 asymptomatic volunteers (age range 21-51 years old, median 23 years old, 14 male and 16 female) with no history of gastrointestinal symptoms, upper gastrointestinal tract surgery, or significant medical conditions. Informed consent was obtained from each subject.

25.1.2 *Patients*

35 patients with IOM based on the Chicago Classification were recruited (age range 18-72 years old, median 40 years old, 15 male and 20 female). This consisted of patients with the following diagnoses: frequent failed peristalsis, weak peristalsis with large peristaltic defect and absent peristalsis. The patients included in this study clinically presented with reflux symptoms (heartburn and/or regurgitation) and/or dysphagia. They were grouped in to two groups according to their predominant symptom to reflux dominant and dysphagia dominant. All patients were requested to stop all medications affecting gastric acid level and oesophageal motility five days prior to their test day. They all completed high resolution manometry and 24 hour impedance-pH monitoring.

Informed consent was obtained prior to any procedure. Patients with history of previous upper GI tract surgeries were excluded from this study.

25.2 High resolution manometry protocol

Manometric studies were done with the patients in the semi-recumbent position after at least a 6-h fast. I used a HRM system with a 32- channel probe (Sandhill HRiM catheter InSight; Sandhill Scientific Inc., Highlands Ranch, CO, USA). Data acquisition, display and analysis were performed using dedicated software (Sandhill Bioview Analysis).

Transducers were calibrated using externally applied pressure. The patients underwent transnasal placement of the manometric assembly and the catheter was positioned to record from the hypopharynx to the stomach. The manometric assembly was positioned with at least 3-5 intragastric sensors to optimize OGJ and intragastric recording. The catheter was then taped to the cheek of the patient. The manometric protocol included a 5-min baseline recording followed by ten 5-ml water swallows.

25.3 HRM analysis

Assessment of the distal oesophageal motility - each swallow was analyzed for integrity of the 20 mmHg isobaric contour (IBC). The final HRM diagnosis for every patient was made according to the 2012 version of the Chicago Classification: ‘weak peristalsis with large peristaltic defects’ if greater than 20% of swallow exhibited large

(>5 cm) breaks in the 20 mmHg IBC, ‘weak peristalsis with small peristaltic defects’ if greater than 30% of swallows exhibited small (2–5 cm) breaks in the 20 mmHg IBC, or ‘frequent failed peristalsis’ if >30% but <100% of swallows were associated with failed peristalsis(59). For the purpose of this study I excluded the patients diagnosed with ‘weak peristalsis with small peristaltic defects’. I defined severe ineffective oesophageal motility (IOM) as ‘weak peristalsis with large peristaltic defects’ and ‘frequent failed peristalsis’. Absent peristalsis was also included in this study.

Assessment of the proximal oesophageal motility - The following parameters were characterize in the proximal oesophagus: (Figure 49)

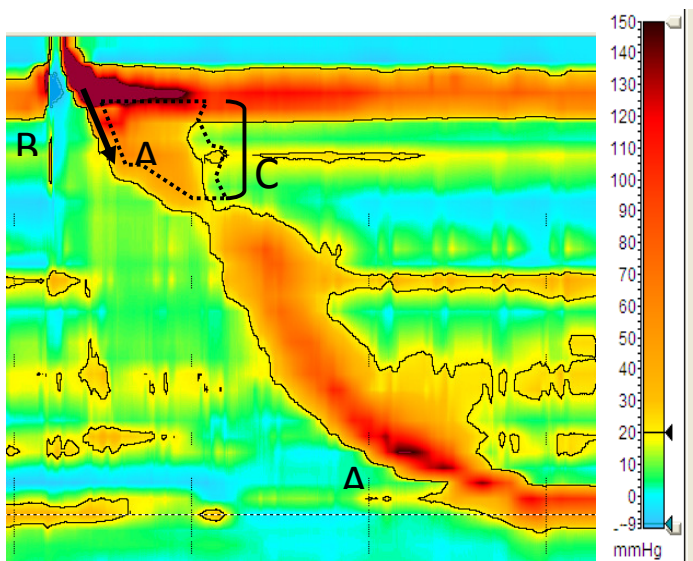


Figure 49 – Quantifying proximal oesophageal parameters – PFV: proximal front velocity, PCI: proximal contractile integral. Isobaric contour is set for 20 mmHg. (image from internal source)

25.4 Length of proximal oesophageal contraction (striated segment of the oesophagus):

Oesophageal peristalsis comprises two distinct contractile waves, corresponding to the distinct muscle types (294, 295). The transition zone represents the region of spatiotemporal merger between these two contractile waves. The length of proximal oesophageal contraction is defined by measuring the peristaltic contraction from the lower border of upper oesophageal sphincter to the beginning of transition zone. This vertical length parameter also corresponds to the length of the striated muscle. An isobaric contour of 20 mmHg is used to delineate the boundaries of the peristaltic wave.

25.5 Proximal Contractile integral (PCI):

Proximal contractile integral is defined as the product of length of peristalsis, duration of peristalsis, and amplitude of the proximal contraction. (mm Hg.cm s). The length of the proximal contraction was decided from the lower border of the UOS up to the beginning of the transition zone using 20 mmHg isobaric contour line.

25.6 Contractile Front Velocity (Proximal Velocity):

The propagation rate of the contractile front through the proximal oesophageal segment is also derived from the isobaric contour plots and characterized as the slope of the line connecting the points on the isobaric contour level of 20 mm Hg calculated in cm/sec. The junction of the peristalsis slope with lower border of the UOS is considered to be at the beginning of the slope, whilst the end of the velocity measurement is considered the point where there is a rapid deceleration of velocity identifiable on the slope of the contractile front velocity.

25.7 Statistical analysis

Establishing the normal values for proximal oesophageal motility: The major parameters included: proximal contractile integral (PCI), proximal peristaltic length (PPL), and proximal contractile front velocity (PFV). Variables are expressed as the mean, standard deviation or as median with range. Fifth and 95th percentile values were calculated and taken as lower and upper limits of the normal variations.

Evaluation of the prevalence and relevance of proximal oesophageal motility in patients with severe distal hypomotility: Proximal contractile integral (PCI), proximal peristaltic length (PPL), and proximal contractile front velocity (PFV) in each of the patients were measured and compared against the normal range from the healthy group. The relation of symptoms and reflux parameters with abnormal proximal motility was assessed. Student's *t*-test or chi-squared analysis was used as appropriate.

26 Results

26.1 Normal values for proximal oesophageal motility

The normal values for proximal oesophagus manometric parameters are shown in (Table17). On average around 300 swallows from 30 asymptomatic healthy volunteers were analysed (age range 21-51 years old, median 23 years old, 14 male and 16 female). Mean of proximal peristaltic front velocity was 7.5 cm/sec with a range of 2.1-16.7 cm/sec. Mean of proximal contractile integral (PCI) was 236.5 mmHg.s.cm with a range of 74.5-420.7 mmHg.s.cm. Mean of proximal peristaltic length was 4.9 cm with a range of 2.6-6.8 cm. (Figure 50 to Figure 52)

Table 17 - Normal values for proximal oesophagus manometric parameters

	PFV cm/sec (proximal front velocity)	PCI mmHg.s.cm (proximal contractile integral)	PPL cm (proximal peristaltic length i.e. Length of
Number of swallows	296	301	303
Minimum	1.6	18	1.7
25% Percentile	3.9	154.6	4.4
Median	5.25	240.1	5.2
75% Percentile	7.675	312.2	5.8
Maximum	82.6	637.4	7.6
5.000% Percentile	2.1	74.51	2.6
95.00% Percentile	16.7	420.7	6.8
Mean	7.517	236.5	4.994

HEALTHY PROXIMAL CONTRACTILE INTEGRAL

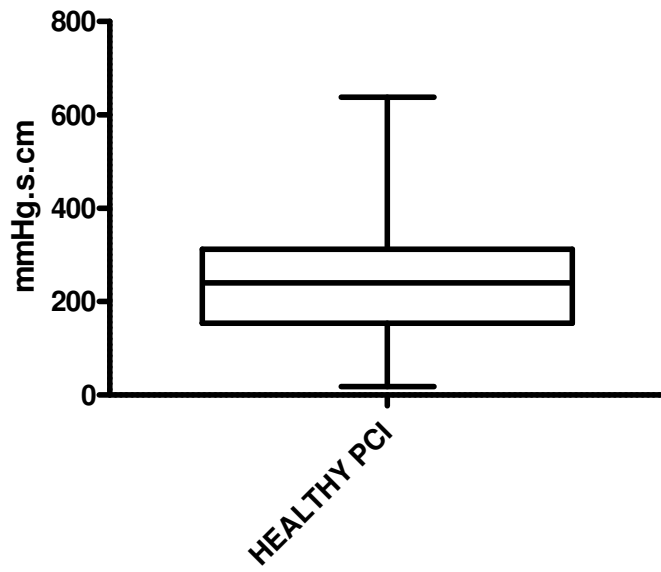


Figure 50

HEALTHY LENGTH OF STRIATED M.

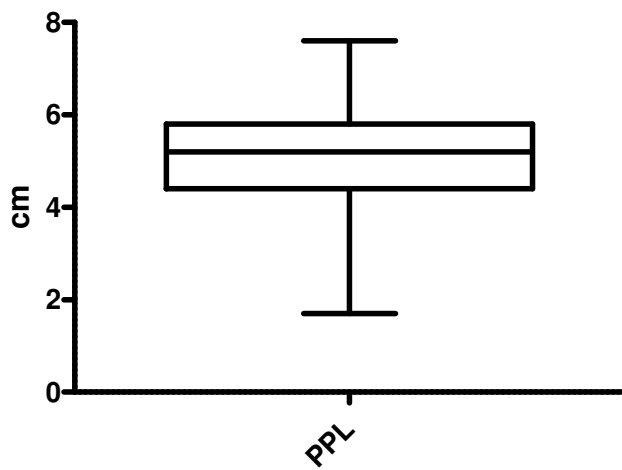


Figure 51

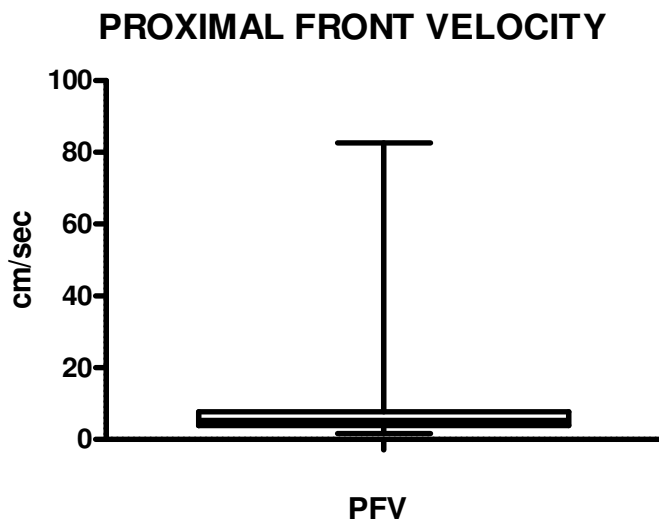


Figure 52

26.2 Prevalence and symptomatology of abnormal proximal oesophageal motility in IOM

In total 35 patients with distal oesophageal hypomotility completed high resolution manometry and reflux monitoring (age range 18-72 years old, median 40 years old, 15 male and 20 female). There was no correlation between the prevalence of oesophageal hypomotility with age or gender in this group of patients.

The criteria to measure the vigor of peristalsis to identify proximal oesophageal hypomotility was PCI. Eleven out of 35 (31%) patients had lower than normal PCI and were hence diagnosed with proximal oesophageal hypomotility 3 with dominantly

dysphagia and 8 with dominantly reflux symptom. Only 2 patients out of those with proximal hypomotility and reflux symptoms had pathological gastro-oesophageal reflux. (Figure 53 to Figure 56) The prevalence of abnormal PFV and abnormal length of striated muscle were 2/35 and 3/35.

Dysphagia and reflux scores were available for 21 patients. The average reflux score was higher in patients with both proximal (based on PCI) and distal oesophageal hypomotility (reflux score 18 versus 13). The average score for dysphagia was lower in patients with both proximal (based on PCI) and distal oesophageal hypomotility (dysphagia score 9 versus 13).

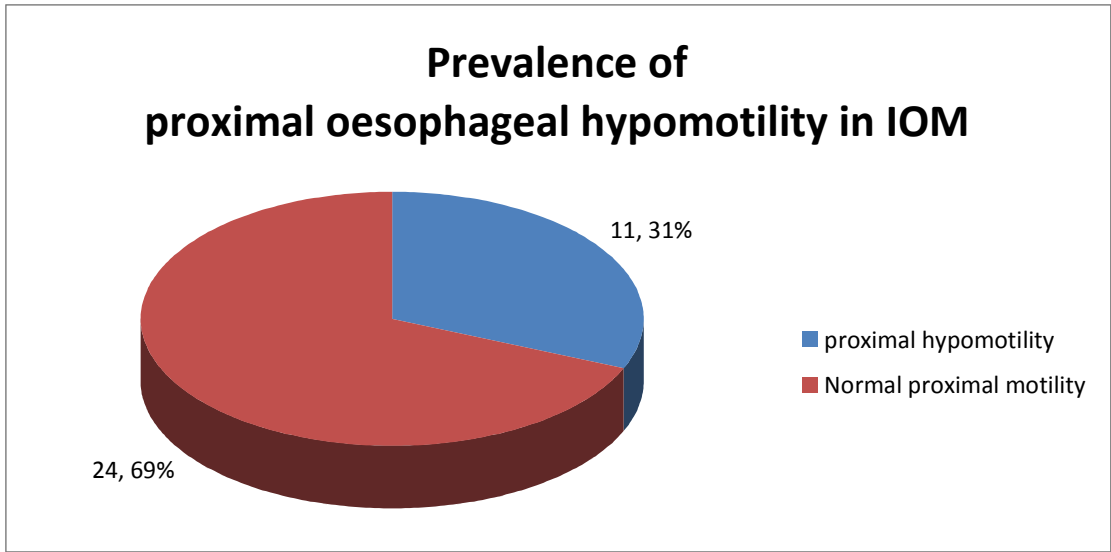


Figure 53

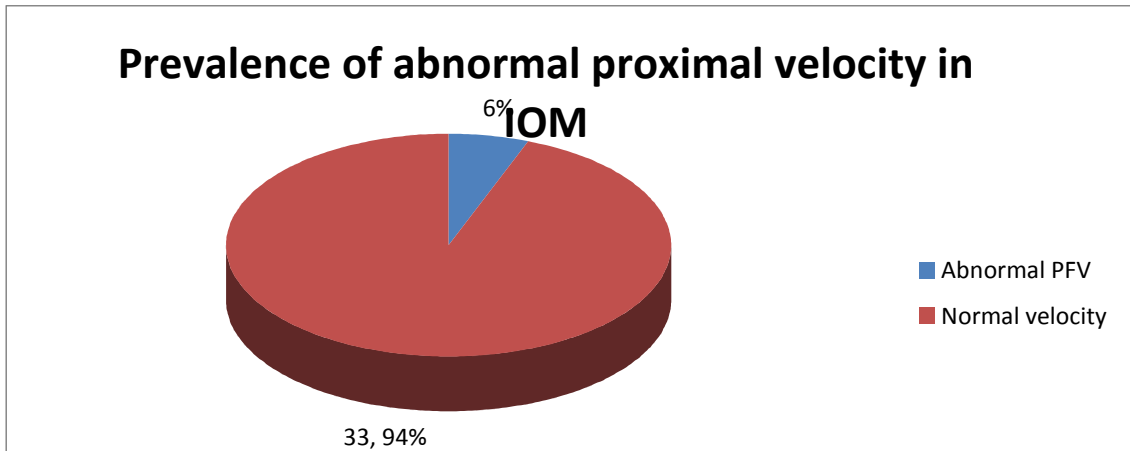


Figure 54

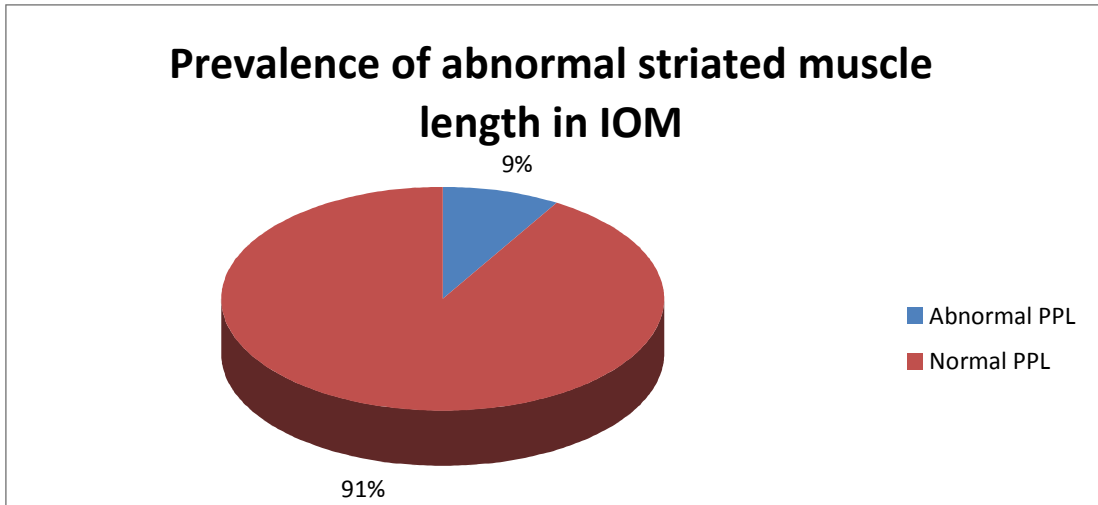


Figure 55

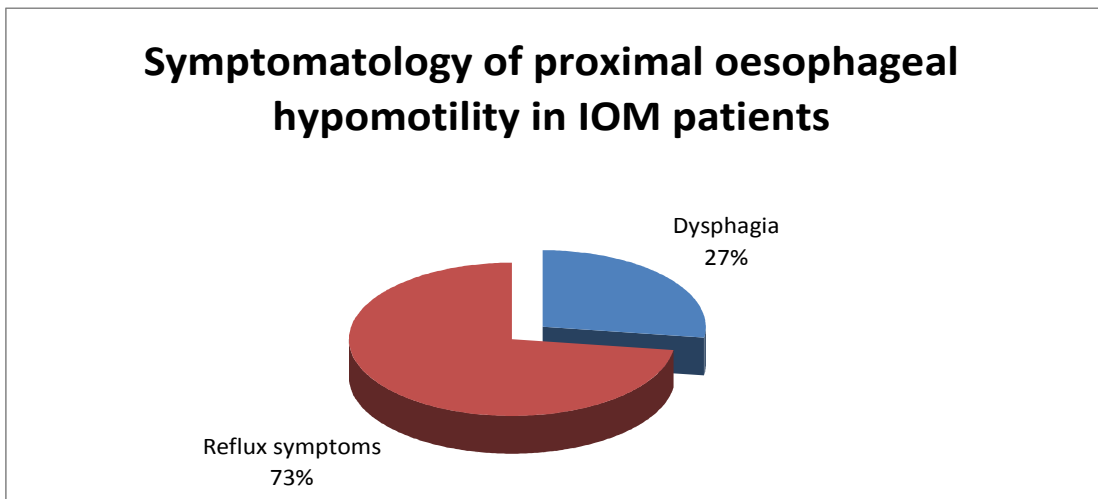


Figure 56

27 Discussion

While significant interest has appropriately been focused on the distal oesophagus and OGJ in the clinical presentation of oesophageal hypomotility, the functional role of the proximal oesophagus has received sparse attention. Historically, this was primarily due to the lack of an appropriate technology to facilitate a detailed segmental analysis of oesophageal peristalsis. The introduction of HRM offers us an opportunity to improve upon this by standardising the testing protocol and facilitating quantitative analysis of proximal oesophageal motility. Such was the first objective of this chapter. With the use of a state-of-the-art HRM probe, I sought to firstly define proximal oesophageal normal motility and secondly define proximal oesophageal hypomotility. Finally, I examined the prevalence of proximal hypomotility in patients with severe distal hypomotility and evaluated the clinical relevance of proximal hypomotility in this group of patients.

Normal ranges for PCI representing proximal motility vigour in our study was significantly smaller than the figures presented by other group (64) (Table: mean PCI of 236.5 mmHg.s.cm versus 779 mmHg.s.cm). This could be mainly due to the use of higher isobaric contour pressure in our study compared to other studies (20 mmHg in our study versus 10 mmHg) which significantly reduces the area of the contractility (please see Figure 48 above).

PFV was also different between our study and previous research study (2.1 – 16.7 cm/s in our study versus 1.9 – 3.8 cm/s). Although the same isobaric contour was detected in both

studies, this difference of proximal velocities particularly in the 95th value could be due to the difference in the selection area for velocity measurement. Similar to the distal peristalsis, there is a two-step contractility pattern in the proximal contractility representing two different velocity figures. Therefore, a proximal deceleration point is identifiable similar to the distal deceleration point. Depending on to which front of the contraction is taken in to account to measure the velocity of proximal peristalsis the PFV might significantly differ. This stepwise deceleration of contraction in the proximal peristalsis is not described in the literature. One explanation could be that structural dynamic of striated muscle is different from smooth muscle having initially very high velocity reaching to a peak and slowing down after the peak point (296). As seen in [Figure 57](#) which compares the force-velocity curve between two types of muscles in general, the velocity of striated muscle follows a different pattern compared to the smooth muscle and our finding of normal ranges for velocity of the striated muscle is compatible with this physiological characteristic of these two types of muscles.

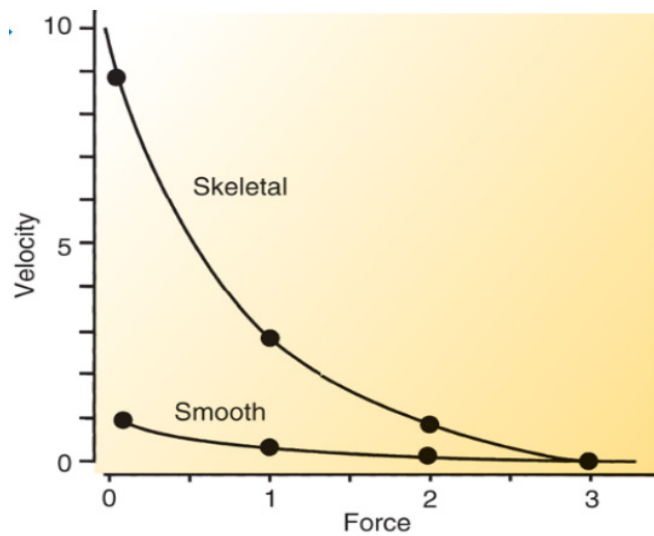


Figure 57 - Skeletal and smooth muscle force–velocity curves. Although the peak forces may be similar, the maximum shortening velocity of smooth muscle is typically 100 times lower than that of skeletal muscle (296).

Another major finding of the analysis was that proximal hypomotility occurred in one third of the patients with severe distal hypomotility. Proximal oesophageal hypomotility can arise due to multiple factors such as weakening of the muscle e.g. myasthenia gravis, mechanisms affecting excitatory cholinergic pathways e.g. anticholinergic medications, or enhanced inhibitory mechanisms ie NO pathway. The majority of the patients with proximal oesophageal hypomotility presented with predominantly reflux symptoms rather than dysphagia. Moreover, the reflux score was higher in patients who presented with oesophageal hypomotility in both proximal and distal oesophagus. In the previous chapter I reported that the patients with severe distal oesophageal hypomotility whose main presentation is reflux symptoms have more widespread oesophageal motility pathways damaged. It is possible that for the same reason, the patients with reflux symptoms also have more defective motility in the proximal oesophagus.

27.1 Conclusion

This is the first study attempting to establish clinical relations between symptoms and hypomotility of the proximal oesophagus.

I characterised the prevalence and clinical relevance of the proximal oesophageal hypomotility in patients with distal oesophageal hypomotility. Weak proximal motility is strongly associated with presentation of the reflux symptoms. These values may prove clinically useful and could contribute to future studies with dysphagic and GORD patients.

CHAPTER 6

TREATMENT OF

OESOPHAGEAL

HYPOMOTILITY:

Effect of Azithromycin on IOM

CHAPTER 6: TREATMENT OF OESOPHAGEAL HYPOMOTILITY: Effect of Azithromycin on IOM

28 Introduction

Ineffective oesophageal motility (IOM) is defined as a swallow response associated with low amplitude contractions and poor bolus transit in the distal oesophagus.

With the advent of high-resolution manometry (HRM) and oesophageal pressure topography IOM is defined as 50% or more swallows with failed or fragmented peristalsis or 2) 50% or more swallows associated with a DCI < 450 mmHg-s-cm²³⁸.

IOM is found in 30% of patients with dysphagia and 20–50% of patients with GORD(69). It is reported that 25% to 55% of patients with oesophagitis have peristaltic dysfunction (297). Therefore, correcting IOM might be beneficial in patients with GORD symptoms or dysphagia.

There is no proven therapy for IOM, but some patients with IOM can improve with treatment of associated reflux disease and/or with prokinetic medications (298). Several pharmaceutical agents known as prokinetic drugs can stimulate GI motility. Prokinetics

like erythromycin, metoclopramide, domperidone and cisapride have been used in adults and paediatric patients (159, 205, 211, 212, 214, 225, 259, 299-304) with variable and somewhat disappointing results. A partial explanation for the disappointing results in clinical practice and clinical trials could be that patients were prescribed prokinetic therapy based on symptoms or reflux monitoring without considering their oesophageal motility status. Theoretically, only patients with significant oesophageal hypomotility would benefit from prokinetic drugs.

Prokinetics may have significant side effects. For example cisapride and tegaserod increase the risk of cardiac arrhythmias and death (305); Bethanechol can cause anxiety, depression, drowsiness, fatigue, involuntary movements and muscle spasms (306); Metoclopramide can cause tardive dyskinesia (306, 307). Prokinetics may induce tachyphylaxis (308, 309). For instance reduced efficacy has been seen with erythromycin after 7-14 days of treatment.

Macrolide antibiotics, such as erythromycin (ERY) have a significant prokinetic effect on proximal gastrointestinal motility via activation of the motilin receptor (206, 303, 310). However, the clinical effectiveness of macrolides for the treatment of chronic GORD - and oesophageal hypomotility - has been hampered by the rapid loss of prokinetic activity due to desensitization of the motilin receptor (311). Azithromycin (AZI) is a macrolide antibiotic with similar in vitro prokinetic effects compared to that observed with erythromycin. Azithromycin stimulates gastric antral activity similar to erythromycin and moreover has a longer duration of effect (approximately 68 hours). However, unlike erythromycin, azithromycin does not have significant drug-drug

interactions(219). Azithromycin is distinguishable due to the long half life and lower drug-drug interactions from the other macrolides/ketolides, despite case reports of cardiac toxicity (312-314). Azithromycin minimally inhibits CYP3A4, which results in the lack of an appreciable interaction with CYP3A4 substrates; thus, azithromycin appears to be the safest macrolide derivative from a cardiac toxicity perspective (315). In a recent publication from the FDA it is recommended to the healthcare providers to be cautious about azithromycin-induced fatal cardiac arrhythmias for patients already at risk for cardiac death and other potentially arrhythmogenic cardiovascular conditions(316). Unlike the short-lasting prokinetic effect of ERY, the effect of AZI on reflux parameters was found several months after start of treatment(214).

Azithromycin has been reported to have clinical efficacy in disorders associated with reduced gastrointestinal motility(219). In a single blinded, placebo-controlled manometric study of 11 healthy patients comparing oral AZI, midecamycin acetate to placebo, Sifrim et al. (317) found that oral AZI 500mg single dose or 250mg b.i.d statistically increased the postprandial antral motility index as compared with placebo. In addition, a recent study showed a statistically significant increased incidence of gastrointestinal side-effects (mainly nausea and diarrhoea) in a group of COPD patients in those on AZI suggesting an AZI-induced gastro-duodenal hypermotility as the cause (318). Finally, a case report using AZI in an elderly patient with diabetic gastroparesis showed symptom improvement after a 3-day treatment with intravenous AZI (319).

28.1 Aim

The aim of this study was to assess the effect of AZI on oesophageal motility in patients with IOM. Secondary outcome measures include the impact of AZI on gastro-oesophageal reflux parameters, gastric emptying, dysphagia and reflux symptoms.

29 MATERIALS AND METHODS

29.1 Subjects and study protocol

Patients referred for oesophageal high resolution manometry who were diagnosed with IOM according to the modified Chicago Classification (236) were selected. 740 patients with IOM were identified from a pool of nearly 5000 patients referred for oesophageal manometry assessments within two centres, Royal London Hospital and Guy's Hospital. Eventually, twenty-six patients fulfilled all the criteria and successfully completed all treatment phases and physiological testing. Patients selected for this study were those who present with one or a combination of dysphagia, heartburn and regurgitation. Excluded patients were those with: 1) motility disorders other than oesophageal hypomotility such as spastic contractions or OGJ obstructions, 2) Barrett's oesophagus larger than 3 cm (endoscopic criteria), 3) hiatus hernia larger than 3cm 4) current cardiac diseases or abnormal ECG performed on each patient before entering the trial study. The

study protocol was approved by the NRES Committee South East Coast - Kent and informed consent was obtained from each subject.

To evaluate the effect of AZI on gastro-oesophageal reflux and gastric motility all patients underwent reflux monitoring with MII-pH and gastric emptying measurements with octanoic acid breath tests before start of the treatment and on the day of taking the last dose of the medication (AZI or placebo).

29.2 High resolution manometry protocol

For details of high resolution manometry protocol see the chapter 2 “methodology”. The manometric protocol in the current study included a 5-min baseline recording, ten 5-ml water swallows, stimulation tests consisted of 3 sets of multiple rapid swallows each of which included 5 x 3ml of water in each set, ten swallows of 5-ml water with externally applied abdominal compression, and 10 swallows of 2 cm³ bread (164).

29.3 HRM analysis

The HRM plot of each swallow was analyzed for integrity of the 20 mmHg isobaric contour. Peristalsis was defined as intact if no break longer than 2 cm was observed in the isobaric contour. Failed peristalsis was defined by minimal (<3cm) integrity of the 20mmHg isobaric contour distal to the proximal pressure trough. When the 20mm Hg isobaric contour was disrupted, the length of the break was measured using the dedicated

tools in each of the analysis software. Fragmented contractions were categorized as contractions with breaks larger than 5cm in the mid oesophagus. The Distal Contractile Integral (DCI in mmHg-s-cm) was calculated as amplitude of the oesophageal contraction (between the transition zone and the LOS) multiplied by the duration and length of peristalsis (59). IOM was defined if 1) 50% or more swallows were followed by failed or fragmented peristalsis or 2) 50% or more swallows triggered contractions with a DCI < 450 mmHg-s-cm (236). Normalising IOM was defined as either reducing the fragmentation to less than 50% of swallows showing >5cm gap in the peristalsis, or less than 50% of swallows show DCI < 450 mmHg-s-cm.

Measurement of LOS baseline pressure was performed using dedicated tool in Bioview Analysis software. The average of end expiratory LOS pressure in at least 3 respiratory cycles in a quiescent area of the tracing (with no effect from swallows or artefacts) was measured.

29.4 Reflux monitoring protocol

For details of reflux monitoring see the chapter 2 “methodology”. The clearance time in this study was measured automatically by the Bioview Analysis software after manually editing the reflux events.

29.5 Gastric emptying

For details of gastric emptying studies see the chapter 2 “methodology”.

29.6 Symptom questionnaire

To evaluate the effect of AZI on symptoms I used the following questionnaires: Dysphagia Odynophagia Questionnaire – This is a validated 10-item questionnaire that assesses the frequency of dysphagia, food impaction and odynophagia. Items are scored from 0-5, using a Likert scale where higher scores represent worse symptoms. A total score out of 50 is calculated – higher scores represent more severe dysphagia. A score of >5 has 86% sensitivity and 97% specificity for identifying the presence of dysphagia (Escobar, Pandolfino et al. 2011).

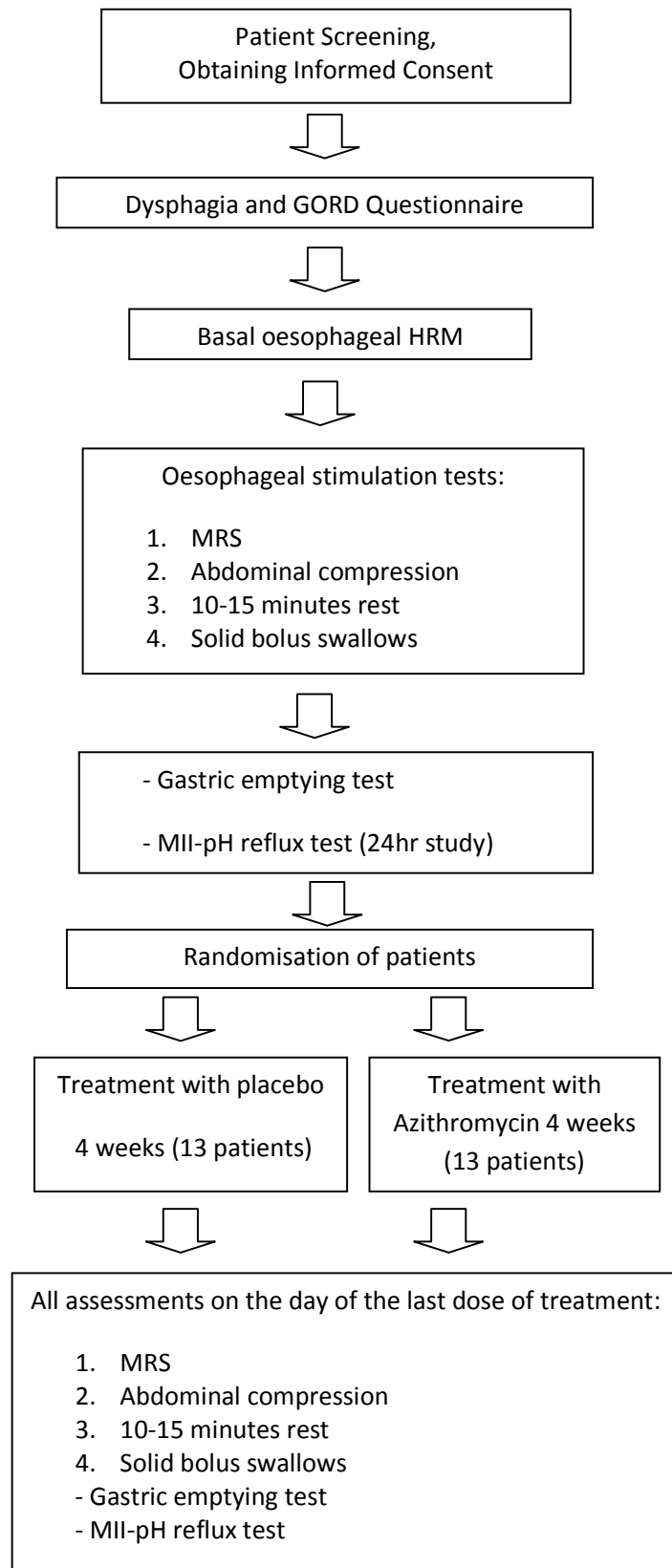
Reflux symptoms were assessed using the Reflux Disease Questionnaire (RDQ) (232) – This is a 12-item self-administered questionnaire, was designed to assess the frequency and severity of heartburn, regurgitation, and dyspeptic complaints and to facilitate the diagnosis of GORD in primary care (232). It scores 12 individual items relating to the frequency and severity of reflux, using Likert scale, where 0 represents the most negative option and 5 the most positive one. A raw score is calculated for domains of heartburn (score: 0-20), regurgitation (score: 0-20) and dyspepsia (score: 0-20), the scores of heartburn and regurgitation can be combined to give a total GORD score (0-40).

Psychological assessment was performed using Hospital Anxiety and Depression Scale (HADS) (233). This is a fourteen item scale that generates ordinal data. Seven of the items relate to anxiety and seven relate to depression. Each item on the questionnaire is scored from 0-3 and this means that a person can score between 0 and 21 for either anxiety or depression.

29.7 Treatment with AZI/placebo

Patients received AZI 250mg orally three times per week on alternate days (214) for four weeks. Identical placebo was administered in the same way. Both placebo and AZI were packed and blinded by the manufacturer (Newcastle Specials, Newcastle upon Tyne, England, UK). Randomization was done by automated software in ratio of 1:1. This study used a double-blind, placebo controlled, parallel design (Figure 58).

Figure 58 – AZI study protocol



29.8 Statistical analysis

The primary outcome measure used in the analysis of the data was the drug-induced change in distal contractile integral (DCI). The cut off level of DCI to diagnose IOM is 450 mmHg.cm.s (236). I expected that the increase of DCI post azithromycin therapy to be at least 50% above the baseline. For the calculation of sample size I considered the level of significance of the test to be 0.05 and the power of the test to be 80%. Using these criteria, the required number of participants in each arm (azithromycin and placebo) was 13 subjects.

Comparisons among the variables from Azithromycin and placebo group were made using contingency tables and non-parametric analysis appropriately (Mann-Whitney analysis when the data is paired and Wilcoxon Signed Rank Test when the data is unpaired). A *P*-value <0.05 was accepted as statistically significant. The data are presented as mean (\pm SD.) or median (\pm interquartile range) as appropriate.

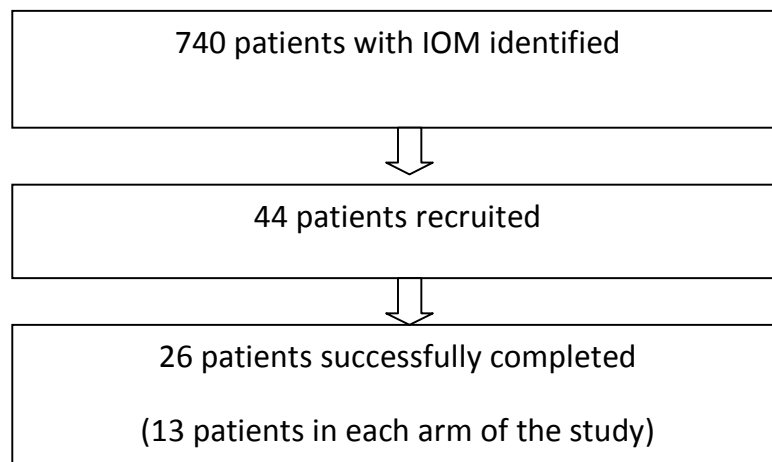
30 RESULTS

30.1 Subjects

Review of our HRM database identified 740 patients with IOM. These patients were contacted and invited to participate in the study. A total of 44 patients were recruited based on their original HRM findings reporting IOM but after entering the study and performing HRM, 19 patients were excluded because they did not have significant

hypomotility. Twenty-six patients [twelve female, median age 54 (25–75 years)] fulfilled the criteria and successfully completed all treatment phases and physiological testing (13 patients in each arm of the study). (Figure 58)

Figure 59 - Number of patients recruited



The HADS (psychological scoring) was used to compare the psychological profile before the treatment between the two groups in order to exclude psychological differences that can influence the effect of drugs on symptoms. HADS score pre-treatment was 5.2 ± 4.7 in AZI group and 9 ± 5.1 in placebo group (NS).

Azithromycin was not associated with any severe side-effects and no patient discontinued the treatment due to side effects. In AZI group loose stool was reported by 1 subject, abdominal cramps by 3 and nausea by 1. In placebo group one patient reported experiencing abdominal cramps and one patient nausea.

Double blindedness of the study should not be affected due to the symptoms as in both arms of the study the participants were guessing to be either on AZI or placebo almost equally.

30.2 Effect of Azithromycin on oesophageal motility (primary outcome)

Azithromycin increased the DCI from 337.7 ± 286.2 mmHg.cm.s to 617.8 ± 384 mmHg.cm.s ($P < 0.01$). Placebo increased the DCI from 374.9 ± 235.9 mmHg.cm.s to 484.4 ± 260 mmHg.cm.s ($P < 0.01$). Comparing the change of DCI against pre treatment session there was no significant difference between AZI and placebo groups ($P < 0.1$). The % increase of DCI after AZI was 162.9 ± 361.2 whereas the % increase of DCI after placebo was 64.5 ± 92.8 .

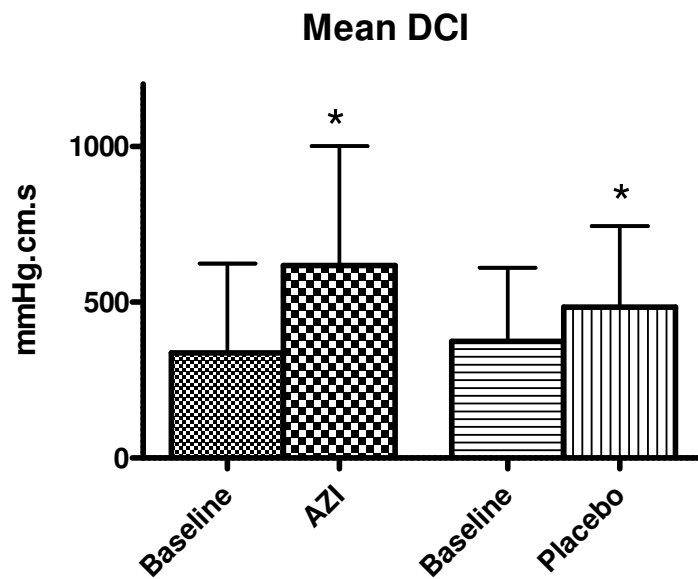


Figure 60 – Comparison of DCI pre and post treatment with AZI and placebo. *Change of DCI against baseline was significant in both group.

30.3 Effect of Azithromycin on IOM

In the AZI group 9 patients had IOM, with DCI <450 mmHg.cm.s. All 13 had fragmented peristalsis. In the placebo group 8 patients had IOM with DCI <450 mmHg.cm.s and 5 with fragmented peristalsis.

In total, AZI significantly changed IOM, either due to normalising the DCI or normalising the fragmented peristalsis. Normalising both DCI and fragmentation together occurred in 4/13 patients in AZI group and none in placebo group.

AZI normalised IOM in 5/9 patients with abnormal DCI whilst placebo did not show such effect in any of the patients with abnormal DCI (P = 0.039).

AZI normalised IOM in 7/13 patients with fragmented peristalsis whilst placebo normalized only 1patient (P< 0.03).

AZI induced >50% increase in DCI in 7/9 patients whilst placebo induced >50% increase in DCI in 4/8 patients (P = 0.4).

Calculation of Odds Ratio and Confidence Interval for the effect of AZI and placebo on normalising IOM: this data confirms the significance of the above findings

	Rate	Risk Ratio	Odds	Odds Ratio	95% Confidence Interval of Odds Ratio
AZI	0.44	0.48	0.8	0.066	Observed = 0.6 Lower limit = 0.005 Upper limit = 0.75
Placebo	0.92		12		

30.4 Effect of AZI on LOS baseline pressure

LOS baseline pressure was increased by AZI from 6.8 (2.3-14.6) mmHg to 11.1 (8.5-16.1) mmHg (P= 0.054). Placebo did not increase LOS pressure [7 (4.7-10.7) mm Hg to 5.2 (2.8-13.3) mm Hg, (P= 0.9)].

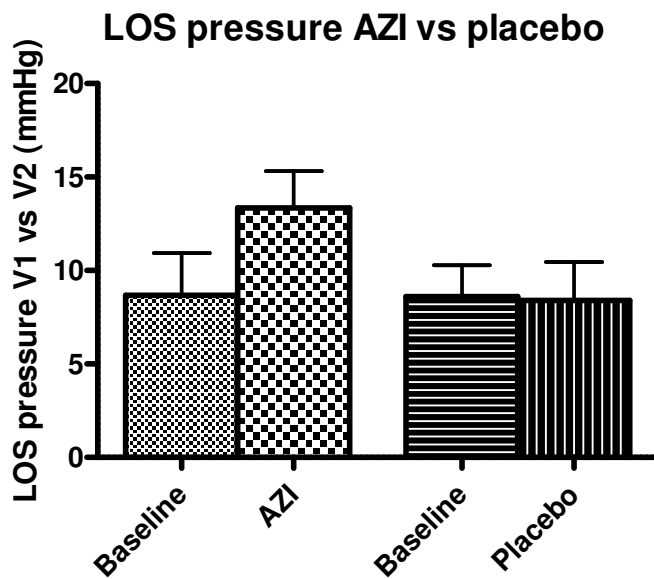


Figure 61 – Comparison of LOS baseline pre and post treatment with AZI and placebo.

30.5 Effect of Azithromycin on gastric emptying

11/26 patients had delayed gastric emptying (5 in the AZI group and 6 in the placebo group). Azithromycin did not affect gastric emptying significantly. During AZI, $t_{1/2}$ decreased from 134.8 ± 35.4 min to 124 ± 34.7 min (means, $P < 0.4$) whilst with placebo the change was from 135.5 ± 22.4 to 144.2 ± 53.3 (means, $P < 0.5$). The number of patients who changed to normal gastric emptying was similar in both groups (AZI: 4 and placebo: 3 patients).

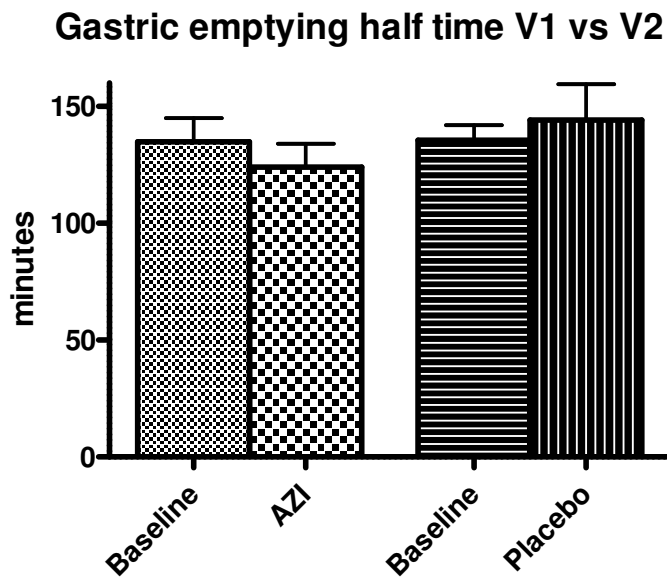


Figure 62 – Comparison of gastric emptying half time pre and post treatments.

30.6 Effect of Azithromycin on gastro-oesophageal reflux

From the 26 patients with IOM that completed the study, only 4 patients had pathological gastro-oesophageal reflux (3 in the AZI group and 1 in the placebo group).

AZI trended to reduce the total number of reflux events (acid and non-acid) from 30.9 ± 20.7 to 20.3 ± 7.1 ($P < 0.09$). AZI trended to reduce the number of acid reflux from 22.4 ± 18.9 to 12.6 ± 7.4 ($P < 0.07$). Placebo did not affect number of reflux episodes [total (34.8 ± 30.5 to 30.4 ± 25.6 , $P < 0.4$) or acid (21.3 ± 22.6 to 21.6 ± 20.1 , $P < 0.9$) reflux].

Total number of reflux events - AZI vs placebo

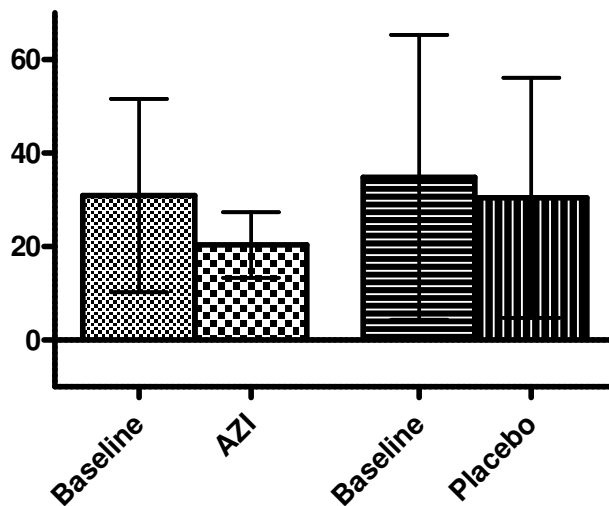


Figure 63 – Comparison of total number of reflux events pre and post treatments.

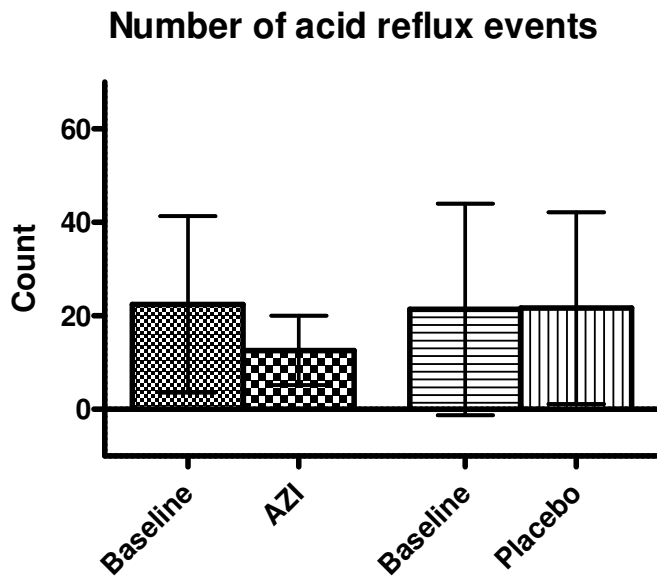


Figure 64 – Comparison of number of acid reflux events pre and post treatments.

Azithromycin trended to reduced reflux volume clearance time from $1.14\% \pm 0.9$ to $0.7\% \pm 0.4$ ($P < 0.1$). Placebo increased clearance time from 1.04 ± 1.1 to 1.2 ± 1.1 ($P < 0.2$).

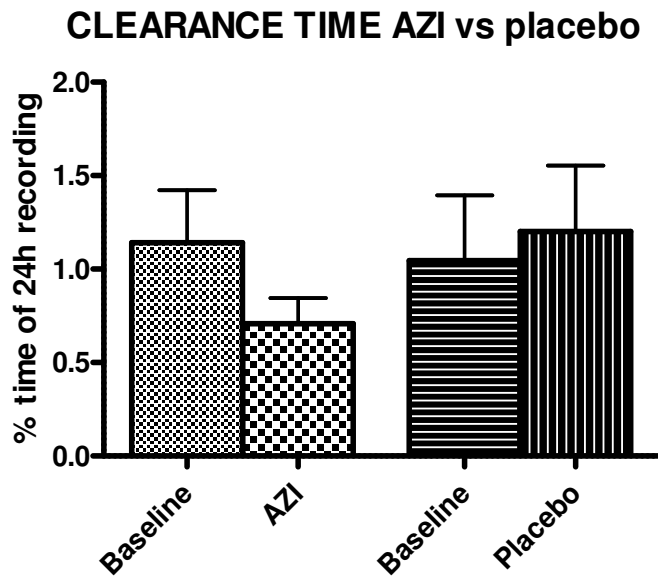


Figure 65 – Comparison of oesophageal clearance time pre and post treatments.

In spite of reducing the number of acid reflux episodes and the clearance time, AZI had no significant impact on acid exposure time (AET) [AET changed from 3.2 (1.4-4.1) to 2 (1.3-3.8); $P < 0.6$].

AET was pathological ($\text{pH} > 4.2$) in 3 patients in the AZI group. In these patients, AZI reduced the number of acid reflux episodes from 57 (41-61) to 22 (16-30) ($P < 0.2$), clearance time from 2.4 (1-3.2) to 0.4 (0.2-1.9) ($P < 0.2$) and acid exposure time from 6.7 (4.2-9.4) to 3.3 (2.1-8.4) ($P < 0.5$). AZI normalised acid exposure in 2/3 patients who had pathological reflux pre-treatment whilst the only patient with pathological acid exposure in the placebo group remained abnormal.

30.7 Effect of Azithromycin on symptoms

Both AZI but not placebo showed significant improvement in the perception of dysphagia. In the AZI group the dysphagia score dropped from 12.45 ± 9 to 9.1 ± 7.4 ($P < 0.01$, 95% confidence interval: 0.8630 to 4.228) whereas in the placebo group the drop was from 10.3 ± 7 to 7 ± 4.1 (4.5-9.5) ($P < 0.06$, 95% confidence interval: 0.1539 to 6.446). The change in dysphagia perception was not significantly different between patients on AZI and patients on placebo ($P = 0.7$).

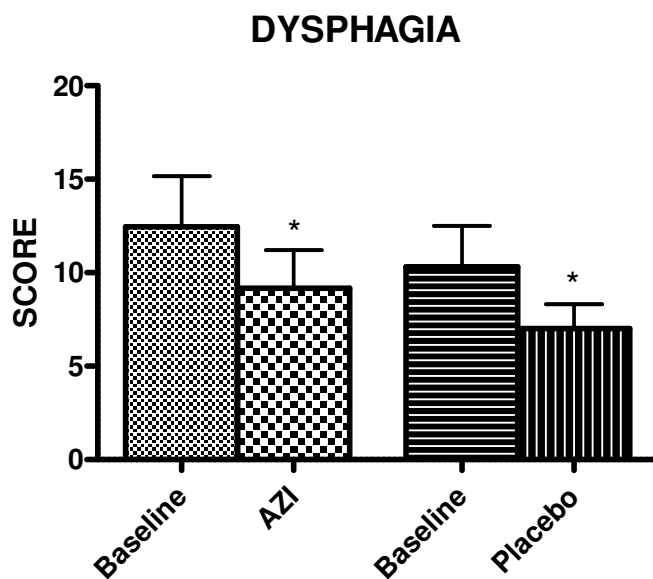


Figure 66 – Comparison of dysphagia score pre and post treatments. (* significant change due to treatment compared to baseline).

Both AZI and placebo showed slight improvement (AZI showed borderline significant effect) in reflux symptoms. In the AZI group the reflux score went from 18.6 ± 13.5 to 14.8 ± 12.6 ($P < 0.049$, 95% confidence interval: -0.02181 to 7.295) whereas in the placebo group the drop was from 21.7 ± 15.3 to 15.8 ± 11.1 ($P < 0.08$, 95% confidence interval: -1.031 to 10.36). There were no statistically differences between treatments.

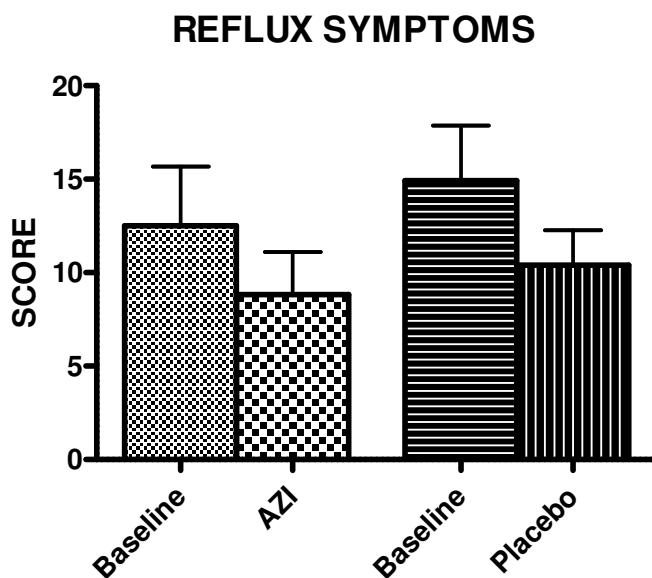


Figure 67 – Comparison of reflux score pre and post treatments.

30.8 Effect of correction of IOM on symptoms

Patients that changed from IOM to non-IOM (either by change in DCI or fragmentation) in the AZI group showed reduction in their reflux scores from 13 ± 11.4 to 9.3 ± 8

(means, $P < 0.07$) and the dysphagia scores from 13.2 ± 9 to 10.7 ± 7.8 (means, $P < 0.01$). Patients who did not change from IOM to non-IOM showed no significant reduction in their reflux scores from 9.3 ± 10.6 to 7.3 ± 8.3 (means, $P < 0.8$) and the dysphagia scores from 14 ± 14.4 to 11 ± 12.9 (means, $P < 0.06$).

31 Discussion

This study assessed the effects of azithromycin on oesophageal body motility in a group of symptomatic patients with IOM.

The main results were: 1. Change of DCI post-treatment with AZI was not significantly different from placebo (although both AZI and placebo changed DCI against baseline DCI in each group). 2. AZI converted IOM status to normal motility in half of the patients whilst placebo did not show such effect. 3. Azithromycin trended to increase LOS pressure 4. AZI did not accelerate gastric emptying. 5. AZI trended to reduce the number of acid reflux events and clearance times. 6. Both AZI and placebo improved the dysphagia and reflux symptoms scores similarly.

To the best of our knowledge there is no previous study describing the effect of azithromycin on oesophageal body motility in human. Most previous studies using prokinetics showed marginal increase in oesophageal body contractions. However, these studies were performed either in healthy subjects or in patients with GORD without severe hypomotility. Our results showed that AZI can improve motility diagnosis in a group of patients with IOM (severe hypomotility) to a normal motility status. This effect

was observed after four weeks of oral treatment whilst most previous studies on the prokinetic effect of this drug were performed after short treatment periods (< 24 hours) (218, 219, 320). The effect of AZI on mean DCI, was larger than that observed with placebo suggesting a potential therapeutic gain which will require further clinical investigation to confirm due to not reaching significant P value. Furthermore, only AZI was able to normalize IOM by increasing DCI or changing the peristaltic pattern.

A significant inter-individual variability in the effect of AZI was observed. The drug-induced increased oesophageal motility was observed in a subgroup of patients with IOM. The reasons for such variability are unclear. Factors involved could be 1. severity or aetiology of the neuromuscular dysfunction, 2. doses of AZI administered or 3. Individual impaired efficacy after 4 weeks of treatment. A test to predict response to AZI would be desirable. I attempted to use provocative tests (pre-treatment) to predict response to AZI. (The results discussed in chapter 7).

It is known that macrolides such as erythromycin have significant prokinetic effect in the oesophagus(67, 321, 322). However, there is lack of clinical efficacy for GORD in longer term use of macrolides (219, 323). In our study AZI showed significant effect on oesophageal motility diagnosis after 4 weeks of treatment. These results are encouraging for the clinical use of AZI in patients with demonstrated oesophageal hypomotility.

AZI trended to increase LOS pressure more than placebo. This effect has been previously shown with other macrolide prokinetics (324).

The pressure increase was not significant (probably due to a type 2 error). In contrast, unlike previous studies (218, 219, 317, 320), I could not observe an effect of AZI on gastric emptying. The reasons for this are unclear. It could be due to the fact that all our patients had normal gastric emptying at baseline making difficult to further accelerate normal emptying rate. Alternatively, the prolonged effect observed in the oesophageal body might have disappeared after 4 weeks of treatment from the stomach.

AZI trended to reduce the number of reflux events and oesophageal clearance time. This effect of azithromycin on reflux parameters has previously been reported by other investigators (214, 325). Our study did not show statistically significant changes in reflux parameters compared to placebo as demonstrated in other studies. This could be due to a type 2 error (the study was powered for the primary outcome i.e. change in DCI). Another explanation could be that very few of our patients had pathological reflux. Nevertheless, acid exposure of 2/3 patients was normalised by AZI whilst the only abnormal patient in placebo group remained abnormal. Alternatively, the effect of AZI on reflux parameters might be due to its effect on proximal gastric motility and position of a postprandial acid pocket (326, 327) (325, 328) rather than an effect on oesophageal motility.

Both AZI and placebo improved the dysphagia and reflux symptoms scores similarly, The sensation of dysphagia was significantly improved in both groups. This is most likely

due to the placebo effect of the whole intervention and the care of patients during the course of this study. Furthermore, a previous study of our group showed a poor correlation between oesophageal motility and bolus transit (measured with impedance) and perception of dysphagia (329).

31.1 Limitations

In the current study I observed a trend or borderline significant results in several parameters which could theoretically become significant by higher number of the participants in this study. Moreover, recruiting more patients with IOM and pathological gastro-oesophageal reflux might help defining the effect of AZI on both reflux parameters and gastric emptying more precisely.

31.2 Conclusion

Our investigation shows that AZI has subtle effects on DCI that did not appear to be as dramatic as it was hoped for. Nevertheless this medication can convert IOM to normal motility to a significantly higher extent compared to placebo. This finding might suggest that, if future studies based on this pilot study can confirm the positive effect of AZI, it can potentially play a role in treatment of conditions associated with IOM such as dysphagia and GORD.

CHAPTER 7

Predictive factors of response to Azithromycin in patients with IOM

CHAPTER 7: Predictive factors of response to Azithromycin in patients with IOM

32 Introduction

Oesophageal peristaltic function is compromised in patients with peptic oesophagitis and/or those presenting with dysphagia (147), with a high incidence of failed peristalsis and hypotensive peristaltic contractions. Oesophageal clearance is compromised significantly when the amplitude of peristaltic contractions in the distal oesophagus falls to values below 25-30 mm Hg (58, 56). With the advent of high-resolution manometry (HRM) and oesophageal pressure topography IOM is defined ²³⁸ as 50% or more swallows with failed or fragmented peristalsis or 2) 50% or more swallows associated with a DCI < 450 mmHg-s-cm. Absent or incomplete peristaltic contractions invariably result in little or no volume clearance. It was demonstrated that a minimal regional peristaltic amplitude is required to prevent retrograde escape of gastric content (56, 330). Correcting this dysfunction might improve symptoms in these patients. Unfortunately, there are no proven treatments for improving oesophageal dysmotility(331).

Several prokinetic agents can stimulate gastrointestinal motility such as erythromycin, metoclopramide, domperidone and cisapride which have been used in adults and paediatric patients with IOM (211) with variable and somewhat disappointing results (331). It is possible that such disappointing results were due to combining patients from

across the GORD or dysphagia spectrum instead of targeting those patients with reversible oesophageal hypomotility. IOM can be due to impaired neuromuscular control of oesophageal motility and/or a structural muscle dysfunction i.e. fibrosis in scleroderma. The pathogenesis and degree of reversibility of IOM in an individual patient would determine response to prokinetic therapy.

I performed a placebo controlled trial on the effect of the macrolide AZI on IOM in patients with dysphagia and GORD (see chapter 6). AZI had a positive effect (compared to baseline and placebo) in a subgroup of patients in whom AZI improved DCI and normalised IOM.

It is not known which factors are able to predict response to prokinetic therapy in patients with IOM. It has been hypothesised that oesophageal stimulation tests could assess the reserve capacity in oesophageal neuromuscular system and hence predict the response to prokinetic therapy. The stimulation tests used in this study. – (multiple swallows of water, increasing outlet resistance at GOJ by applying abdominal compression, bread swallows) - use different pathways to stimulate oesophageal contraction. Multiple rapid swallows particularly requires intact deglutitive inhibition and preserved excitatory mechanism to provoke after MRS contraction (281). Bread swallows requires preserved afferent pathway to detect the presence of the solid bolus in the oesophageal body and effective excitatory efferent pathway to enhance the strength and coordination of oesophageal contractions (282). Abdominal compression requires an intact mechanism

that detects increased resistance at the GOJ level and increases excitatory pathways to augment the strength of contraction to overcome the resistance and assure oesophageal emptying (234, 256, 263, 283).

In this chapter, I assessed the predictive value of the stimulation tests for positive response to Azithromycin in patients with ineffective oesophageal motility

33 Materials and methods

33.1 Patients

In the AZI group 9 patients had IOM with DCI <450 mmHg.cm.s. The other 4 patients were included based on presence of fragmented peristalsis.

AZI normalised IOM in 5/9 patients with abnormal DCI and corrected fragmented peristalsis in 8/13 patients. When both DCI and fragmentation criteria was combined, AZI normalised 8/13 patients.

For the purpose of assessment of predictive factors of positive response to AZI, I performed 2 analyses. First, I considered as responders only those 5 patients that had significant increase in DCI. Second, I considered responders all patients that normalized IOM i.e. increased DCI and/or improved fragmented peristalsis.

33.2 Predictive value of the stimulation tests

I determined the average baseline DCI before provocative tests (during single water swallows) and the DCI at the after-contraction following multiple rapid swallows; during bread swallows and during water swallows under abdominal compression. Thereafter, I calculated a provocative test/wet swallow DCI ratio.

A ROC analysis was used to determine the threshold for each parameter that best discriminated patients with good response to AZI. Thereafter, I calculated the sensitivity, specificity, predictive values and likelihood ratios.

34 Results

The first analysis considered as responders all patients that improved their motility with AZI to a non-IOM condition i.e. 8/13 patients had significant increase in DCI and/or normalised the peristaltic fragmentation.

ROC analysis showed the following results:

The best threshold value for DCI after MRS was ≥ 248 mmHg.cm.s. This cut off value could segregate responders to AZI from non-responders with a sensitivity of 78%, specificity of 75%, PPV of 87.5%, NPV of 60% and the LR of 3.11.

The ability of DCI to identify patients responders to AZI during bread swallows and abdominal compression was lower than DCI after MRS and are shown in table 18.

Combining the criteria for DCI after MRS and DCI during abdominal compression yielded in a sensitivity of 67% and negative predictive value of 80%.

In a second analysis, the ratio of the DCI of each stimulation test/baseline DCI was calculated. The optimum cut off value for the ratio of DCI after MRS was >1.2. This cut off value could segregate responders to AZI from non-responders with a sensitivity of 78% , specificity 75%, PPV 87.5%, NPV 60% and the LR of 3.11.

The ability of DCI ratios to identify patients responders to AZI during bread swallows and abdominal compression was lower than DCI ratios after MRS and are shown in table18.

In a third analysis I considered as responders only those 5 patients who had significant increase in DCI with AZI to a non-IOM condition.

The best threshold value for DCI after MRS was ≥ 395 mmHg.cm.s. This cut off value could segregate responders to AZI from non-responders with a sensitivity of 80%, specificity 75%, PPV was 80%, NPV 75% and LR 3.2.

The best cut-off value for DCI during bread swallows was >296mmHg.cm.s. Using this cut off, the sensitivity was 60 the specificity was 50, the PPV was 60 the NPV was 50 and the LR was 1.2.

The best cut-off value for DCI during abdominal compression was >587 mmHg.cm.s.

Using this cut off, the sensitivity was 80% the specificity was 75%, the PPV was 80% the NPV was 75% and the LR was 3.2.

Combining the criteria for DCI after MRS and DCI during abdominal compression increased sensitivity and negative predictive value to 100%.

Table 18 – Predictive value of the stimulation tests

Predictive value of the stimulation tests based on DCI and peristaltic fragmentation of oesophageal response to tests (Pure DCI values)							
Pure DCI of stimulation tests	Sensitivity %	Specificity %	Likelihood ratio	Positive predictive value %	Negative predictive value %	Area under ROC curve	
Multiple rapid swallow DCI (≥ 248 mmHg.cm.s)	78	75	3.11	87.5	60	0.6	
Bread swallow DCI (<565mmHg.cm.s)	60	33.3	0.9	75	20	0.7	
Swallow with abdominal compression DCI (>769 mmHg.cm.s)	80	50	1.6	50	80	0.65	
Combining MRS and abdominal compression	67	40	1.11	25	80	na	

Predictive value of the stimulation tests based on DCI and peristaltic fragmentation of oesophageal response to tests (Ratios)						
Ratio of stimulation test/baseline swallow DCI	Sensitivity %	Specificity %	Likelihood ratio	Positive predictive value %	Negative predictive value %	Area under ROC curve
Multiple rapid swallow/ baseline (>1.2)	78	75	3.11	87.5	60	0.67
Bread swallow / baseline (<2.1)	56	25	0.7	62.5	20	0.72
Swallow with abdominal compression / baseline (>3.9)	80	50	1.6	50	80	0.57
Combining MRS and abdominal compression	100	50	2.25	50	100	na
Predictive value of the stimulation tests based on DCI of oesophageal response to tests						
	Sensitivity %	Specificity %	Likelihood ratio	Positive predictive value %	Negative predictive value %	Area under ROC curve
Multiple rapid swallow DCI (≥ 395 mmHg.cm.s)	80	75	3.2	80	75	0.85
Bread swallow DCI (>296mmHg.cm.s)	50	20	0.6	50	20	0.5
Swallow with abdominal compression DCI (>587 mmHg.cm.s)	67	50	1.33	75	40	0.75
Combining MRS and abdominal compression	100	67	3	60	100	na

35 Discussion

In the current chapter, I assessed the oesophageal body response to stimulation tests to identify patients with clear oesophageal hypomotility who are more likely to respond to AZI therapy. I hypothesized that such stimulation tests could reveal the degree of preserved contractile capacity in patients with ineffective oesophageal motility (255-258, 332). Theoretically, those patients with preserved contractile capacity would be the responders to prokinetic therapy with AZI.

I analysed the contractile response to AZI in 3 different ways. First I considered responders all patients that normalized the IOM (increasing their DCI and/or improving peristaltic fragmentation). Second, I calculated a ratio between DCI after the tests and DCI at baseline and I used such ratio to segregate patients. Finally, I only consider as responders those patients that normalized the DCI (without considering fragmentation).

The general results of these analysis showed that the predictive value of stimulation tests is moderately good (the areas under ROC curves were between 0.5 and 0.85 and the likelihood ratios were between 0.7 and 3.2). The best predictors were the absolute value of DCI after MRS and during abdominal compression. Furthermore, combining these two stimulation tests increased the negative predictive value of the tests. Interestingly, the worst predictor was the DCI during bread swallows.

Previous studies have shown that multiple rapid swallowing is a reliable maneuver to test the reserved neuromuscular oesophageal capacity (255, 256). Studies by Gyawali et.al, using a similar analysis of DCI before and after fundoplication, concluded that multiple rapid swallowing has a predictive value to identify patients that will develop late dysphagia after anti reflux surgery (255, 258). In the current study, I found that patients that responded to AZI had higher contractile response to provocative tests such as MRS. Previous studies have reported the effect of increased oesophageal outlet resistance on generating an immediate increase of DCI (333). Our study suggests that presenting a more vigorous response to abdominal compression can also be interpreted as having sufficient reserved capacity to respond to AZI.

Interestingly, analysis of combined MRS and abdominal compression provided a very high negative predictive value, suggesting that a patient with oesophageal hypomotility that is unable to increase contractility after MRS and abdominal compression is unlikely to respond to prokinetic therapy such as AZI or other similar drugs.

A significant limitation of this study is the small number of patients analysed. It is possible that the results are influenced by such small number. Therefore, I still consider these results as preliminary and will need to increase the number of patients to provide more definite conclusions.

The low likelihood ratios that I observed with our tests suggest that other parameters such as duration and severity of IOM, age, BMI etc. might be involved and could have an impact on response to AZI.

In conclusion, our preliminary results suggest that provocative tests such as multiple rapid swallowing and/or swallows with abdominal compression can assess oesophageal peristaltic reserve in the oesophageal body. Assessment of such reserve capacity might have clinical value during evaluation of patients with IOM and, may help predicting the response of these patients to prokinetic therapy.

Chapter 8

General discussion and future prospects

Chapter 8: General discussion

IOM is a common diagnosis in upper GI physiology units in general. Smout et al reported OH with or without hypotensive LOS in 58% of 2610 patients referred for oesophageal manometry(66) (they used the term OH which is a more generic term than IOM). Others reported IOM in 27–32% of patients presenting with non-obstructive dysphagia without GORD(67-70). It is also the most prevalent oesophageal motor disorder in GORD, found in 21–38% of patients in large series(69, 71-73).

Pathogenesis of IOM is not completely understood. IOM can be secondary to other diseases or a primary entity. Impaired cholinergic stimulation is considered to be the main defect underlying IOM (90). Few myopathic pathologies such as Progressive Systemic Sclerosis can produce oesophageal hypomotility(91). IOM can be observed in patients without evidence of GORD or connective tissue disorders. It is suggested that patients with IOM may have an imbalance between excitatory and inhibitory neural activity in the oesophageal body, due to an abnormal ratio between choline acetyltransferase (ChAT) and nitric oxide synthase (nNOS) by neurons in the myenteric plexus(91).

In GORD, inflammatory mediators, such as interleukin-6 and platelet-activating factor, produced during acute oesophagitis, can reduce acetylcholine release from excitatory myenteric neurons to circular smooth muscle(89, 106, 107).

Symptoms associated with IOM include dysphagia, odynophagia, heartburn and regurgitation. Also, extra-oesophageal symptoms such as cough(146), globus and hoarseness are attributed to oesophageal hypomotility. However, there is no clear agreement between objective measurements of oesophageal function and subjective perception of bolus passage i.e. dysphagia. Increased bolus passage perception in patients without mechanical obstruction might be due to oesophageal hypersensitivity (329). Therefore, symptom based diagnosis is not reliable in patients with swallowing problems, heartburn and other dyspeptic complaints (154, 155).

Oesophageal manometry is considered the gold standard for diagnosis of IOM. Using high-resolution manometry IOM is defined²³⁸ as 50% or more swallows with failed or fragmented peristalsis or 2) 50% or more swallows associated with a DCI < 450 mmHg.cm.s. HRM cannot distinguish the underlying cause of the oesophageal hypomotility i.e. structural i.e. scleroderma versus neural imbalance i.e. GORD.

In the process of developing treatment strategies in oesophageal hypomotility, testing the potential reversibility of IOM in patients could be useful to predict the response of these patients to new treatments and prokinetic drugs. Provocative tests during HRM include multiple rapid swallows (MRS), solid bolus swallows (252), and abdominal compression test. Responses to provocative/stimulation tests may be useful to assess the reserve

capacity of oesophageal motility (253). For example, multiple rapid swallows test may predict the likelihood of postoperative dysphagia in patients undergoing antireflux surgery(254, 255).

IOM can improve with treatment of associated reflux disease and/or with prokinetic medications (Fornari et al 2007). Prokinetics like erythromycin, metoclopramide, domperidone and cisapride have been used in adults and paediatric patients (159, 205, 211, 212, 214, 225, 259, 299-304) with variable and somewhat disappointing results. A partial explanation for the disappointing results in clinical practice and clinical trials could be that patients were prescribed prokinetic therapy based on symptoms or reflux monitoring without considering their oesophageal motility status. Theoretically, only patients with significant oesophageal hypomotility would benefit from prokinetic drugs.

The research studies that conform this PhD thesis aimed to establish a methodology for identification of patients with sufficient reserved oesophageal neuromuscular functionality to respond to prokinetic therapy. Therefore, I aimed to: 1. assess IOM in patients with non-obstructive dysphagia and GORD, using HRM; 2. develop and standardize a set of “stimulation tests” to assess degree of IOM reversibility, 3. treat IOM with a prokinetic agent and assess the outcome on oesophageal motility, dysphagia and GORD; 4. evaluate the role of stimulation tests in predicting manometric and clinical to prokinetic therapy in patients with IOM.

36 High resolution manometry

The evaluation of oesophageal motor function may be influenced by the manometric system employed. HRM can be performed with devices manufactured by different companies. I first established the normal range for high resolution manometry values using a Sandhill-Unisensor AG assembly and compared our data with the values published by the consensus Chicago group using the Sierra Scientific Instruments system (59).

Our findings from this comparison revealed that the normal values of the parameters investigated with Unisensor AG assembly had some differences from those of the previous studies that used the MonoScan system. While the normal values for DCI in our study were similar to the data previously established by Chicago Classification, the CFV and DL cut offs were slightly different. Even more importantly, the upper cut off for IRP in the supine position utilized in the Chicago Classification is 15 mmHg (248), whereas according to our results with the Unisensor AG HRM device it should be 23.5 mmHg. This is a particularly important issue because IRP is a key measurement in the HRM criteria to decide on the completeness of the LOS relaxation during diagnosis of achalasia (249).

Our results, taken together with those of Smout et al (247) and of Shi et al, indicated that HRM systems using the Unisensor AG catheter provides consistently higher IRP values

in healthy volunteers than those of ManoScan system used to establish the Chicago classification.

37 Oesophageal stimulation tests

Current diagnostic classifications for conventional and HRM are based on repeated small volume water swallows in the supine position (55, 248). Some authors have recommended performing stimulation tests such as solid swallows (164), to increase sensitivity to symptomatic dysmotility and dysfunction; In our studies I established normal values for HRM parameters of peristaltic and OGJ function during stimulation tests.

The mean of the differences in DCI produced by each of the stimulation tests were compared against single water swallows. All three stimulation tests were able to induce DCIs significantly higher than single swallows of water.

MRS yielded the lowest IRP amongst all the swallowing tests. Multiple swallows provide enough time for the LOS to relax completely and there is no increased intrabolus pressure as in bread swallows. From previous studies (164, 256, 273) it was expected an increase in IRP during bread swallows (61). Our study confirmed this concept.

Effects of bolus consistency and load on oesophageal function were consistent with previous studies using conventional and high-resolution manometry (159, 274, 275). As expected from previous studies (164), as workload increased, oesophageal contractile

response was slower [lower contraction front velocity (CFV)], better coordinated (shorter transition zone) and more vigorous [greater distal contractile integral (DCI)].

The peristaltic break i.e. transition zone during stimulation tests was shorter than during single water swallows. This was more pronounced during bread swallows.

MRS was found to be the stimulation test with the highest provocative capacity to induce stronger contractility in the oesophageal body.

Overall reproducibility of stimulation tests in oesophageal HRM data was good. There was minimal difference between inter and intra-individual %COV. In addition, concordance testing showed that significant but not perfect concordance values were found for all stimulation tests. This supports the use of stimulation tests as additional tools in the clinical evaluation of the patients with oesophageal motor disorders in order to assess the reserved oesophageal neuromuscular capacity.

What are the potential mechanisms that trigger increased contractions during stimulation tests? Oesophageal mechano-receptors (stretch sensors) are probably initially implicated in the 3 stimulation tests. In the outlet obstruction induced by abdominal compression, the increased resistance against the passage of bolus and peristaltic propelling pressure provokes increased wall expansion and stretch. Such stimulus can trigger a peripheral reflex (in the oesophageal wall) resulting in increased circular and longitudinal smooth muscle contraction in the segment above the bolus via a cholinergic, muscarinic mechanism (285, 286). Furthermore, changes either in the preload (muscle length or stretch) or afterload (the mass of the bolus) induces contraction with higher amplitude

(287, 288). Finally, pressure sensors in the abdominal cavity or stomach might stimulate the afferent limb of a vago-vagal reflex arch modulating oesophageal peristalsis(287, 289).

Similar mechanisms can explain the effect of bread swallows. However, the after contraction at the end of multiple rapid swallowing probably requires an additional central input from central nervous system. During repetitive swallows, there are central inhibitory signals transmitted to the oesophageal body causing hyperpolarisation of the smooth muscle cells leading to a strong after-contraction (290).

In normal subjects, the different stimulation tests used in this study were able to trigger increased oesophageal contractility. Peripheral and central pathways are involved. The MRS uses more central and the abdominal compression and bread swallows more peripheral pathways. In our study I used the tests to assessed reserve capacity of patients with severe hypomotility. I identified a group of patients with appropriate reserve capacity (hypomotility in basal condition and increased contractility during stimulation).

I could not demonstrate significant differences in response to stimulation tests between IOM patients with reflux or presenting with dysphagia. However, I identified a trend towards a more severe failure i.e. worse reserve capacity in reflux patients compared to patients with dysphagia.

I hypothesized that a distinct response to stimulation test would predict the predominant symptom. However, our findings did not support such a hypothesis. There was no clear difference in response to stimulation tests between patients with predominant reflux symptoms compared to patients with predominant dysphagia.

More than one third of patients with reflux symptoms and pathological acid gastro-oesophageal reflux (37,5%) had all three stimulation tests abnormal whilst this finding was the least frequent in the dysphagia group (10%) (it should be noted that I had only 34 patients studied in this section).

Based on this trend, I could speculate that the mechanism underlying oesophageal hypomotility in reflux disease is different from that in dysphagia. Tutuian et al reported that a higher proportion of oesophageal motility abnormalities during bread swallows was observed in patients with chest pain and GERD symptoms compared to patients with dysphagia (284).

The IRP in patients with dysphagia was slightly higher than in the reflux group suggesting that increased intrabolus pressure associated with a higher distal resistance might be more relevant to dysphagia sensation than the severe hypomotility alone. In contrast, patients with lower IRP (reflux group) had more abnormal response to all three stimulation tests, suggesting that a better motility response to stimulation tests has less impact in dysphagia perception.

38 Proximal hypomotility and clinical presentation of IOM patients

While significant interest has appropriately been focused on the distal oesophagus and OGJ in the clinical presentation of oesophageal hypomotility, the functional role of the proximal oesophagus has received sparse attention. Historically, this was primarily due to the lack of an appropriate technology to facilitate a detailed segmental analysis of oesophageal peristalsis. The introduction of HRM offers us an opportunity to improve upon this by standardising the testing protocol and facilitating quantitative analysis of proximal oesophageal motility. With the use of a state-of-the-art HRM probe, I sought to firstly define proximal oesophageal normal motility and secondly define proximal oesophageal hypomotility. Finally, I examined the prevalence of proximal hypomotility in patients with severe distal hypomotility and evaluated the clinical relevance of proximal hypomotility in this group of patients.

Normal range for proximal contractile integral (PCI) representing proximal motility vigour in our study was significantly smaller than the figures presented by other groups (64). This could be mainly due to the use of higher isobaric contour pressure in our study compared to other studies (20mmHg vs 10mmHg) which significantly reduce the area of the contractility. When higher threshold is selected, the area under isobaric contour will be reduced.

Proximal hypomotility occurred in one third of patients with severe distal hypomotility. Proximal oesophageal hypomotility can arise due to multiple factors such as weakening of the muscle e.g. myasthenia gravis, mechanisms affecting excitatory cholinergic pathways e.g. anticholinergic medications, or enhanced inhibitory mechanisms ie NO pathway. The majority of the patients with proximal oesophageal hypomotility presented with dominantly reflux symptoms rather than dysphagia. The reflux score was higher in patients who presented with oesophageal hypomotility in both proximal and distal oesophagus.

39 Treatment of IOM with Azithromycin

I investigated the effects of azithromycin on oesophageal body motility in a group of symptomatic patients with IOM.

The main results were: 1. there is an indication that Azithromycin may significantly increase oesophageal body motility (DCI). 2. AZI converted IOM status to normal motility in half of the patients whilst placebo did not show such effect. 3. Azithromycin trended to increase LOS pressure 4. AZI did not accelerate gastric emptying. 5. AZI trended to reduce number of acid reflux events and clearance times. 6. AZI improved the dysphagia and reflux symptoms scores similar to placebo.

To the best our knowledge there is no previous study describing the effect of azithromycin on oesophageal body motility in humans. Most previous studies using

prokinetics showed marginal increase in oesophageal body contractions. However, these studies were performed either in healthy subjects or in patients with GORD without severe hypomotility. Our results showed that AZI can improve motility in a group of patients with IOM to a normal motility status. This effect was observed after four weeks of oral treatment whilst most previous studies on the prokinetic effect of this drug were performed after short treatment periods (< 24 hours) (218, 219, 320). The effect of AZI on DCI, was larger than that observed with placebo suggesting a potential therapeutic gain. Furthermore, only AZI was able to normalize IOM by increasing DCI.

I observed a significant inter-individual variability in the effect of AZI. The drug-induced increased oesophageal motility was observed in a subgroup of patients with IOM. The reasons for such variability are unclear. Factors involved could be 1. severity or aetiology of the neuromuscular dysfunction, 2. doses of AZI administered or 3. individual impaired efficacy in longer term treatment.

It is known that macrolides such as erythromycin have significant prokinetic effect in the oesophagus (67, 321, 322). However, there is lack of clinical efficacy for GORD in longer term use of macrolides (219, 323). In our study AZI showed indication of possible significant effect on oesophageal motility after 4 weeks of treatment. These results are encouraging for the clinical use of AZI in patients with demonstrated oesophageal hypomotility.

AZI trended to increase LOS pressure more than placebo. This effect has been previously shown with other macrolide prokinetics (324). The pressure increase was not significant (probably due to a type 2 error). In contrast, unlike previous studies(218, 219, 317, 320), I could not observe an effect of AZI on gastric emptying. The reasons for this are unclear. It could be due to the fact that all our patients had normal gastric emptying at baseline making difficult to further accelerate normal emptying rate.

AZI trended to reduce the number of reflux events and oesophageal clearance time. The significant effect of Azithromycin on reflux parameters has previously been reported by other investigators(214, 325). Our investigation did not show statistically significant changes in reflux parameters compared to placebo as was demonstrated in other studies. This could also be due to type 2 error (the study was powered for the primary outcomes i.e. change in DCI). Another explanation could be that very few of our patients had pathological reflux. Nevertheless, acid exposure of 2/3 patients was normalised by AZI whilst the only patient with abnormal acid exposure in the placebo group did not benefit from AZI. Alternatively, the effect of AZI on reflux parameters might be due to its effect on proximal gastric motility and position of a postprandial acid pocket (326, 327) (325, 328) rather than an effect on oesophageal motility.

Our investigation which is a pilot study shows that AZI may convert IOM to normal motility to a significantly higher extent compared to placebo. This finding might suggest

that AZI can potentially play a role in treatment of conditions associated with IOM such as dysphagia and GORD.

40 Predictive value of stimulation tests for treatment of IOM with AZI

I assessed the oesophageal body response to stimulation tests to identify patients with OH who are more likely to respond to AZI therapy. Our hypothesis was that such stimulation tests could reveal the degree of preserved contractile capacity in patients with ineffective oesophageal motility (255-258, 332). Theoretically, those patients with preserved contractile capacity would be more likely to respond to prokinetic therapy with AZI.

The predictive value of stimulation tests was moderately good. The best predictors were the absolute value of DCI after MRS and during abdominal compression. Furthermore, combining these two stimulation tests increased the negative predictive value of the tests. Interestingly, the worst predictor was the DCI during bread swallows.

Previous studies have shown that multiple rapid swallowing is a reliable manoeuvre to test the reserve neuromuscular oesophageal capacity (255, 256). Studies by Gyawali et al, using a similar analysis of DCI before and after MRS, concluded that multiple rapid swallowing is able to identify patients who will develop late dysphagia after anti reflux

surgery (255, 258). I found that patients that responded to AZI had higher contractile response to provocative tests such as MRS. Previous studies have reported the effect of increased oesophageal outlet resistance on generating an immediate increase of DCI (333). Our study suggests that presenting a more vigorous response to abdominal compression can also be interpreted as having sufficient reserved capacity to respond to AZI.

Interestingly, analysis of combined MRS and abdominal compression provided a very high negative predictive value, suggesting that a patient with OH that is unable to increase contractility after MRS and abdominal compression is unlikely to respond to prokinetic therapy such as AZI or other similar drugs.

The low likelihood ratios that I observed with our tests suggest that other parameters such as duration and severity of IOM, age, BMI etc. might be involved and could have an impact on response to AZI.

Our preliminary results suggest that provocative tests such as multiple rapid swallowing and/or swallows with abdominal compression may have clinical value during evaluation of patients with IOM and may potentially predict the response to prokinetic therapy.

41 Future prospects

The research projects presented in this thesis aimed to study different aspects (pathophysiology, diagnosis and treatment) of ineffective oesophageal motility (IOM). IOM is known to be prevalent in patients with GORD and/or dysphagia. However its role in pathophysiology of these conditions is not well established neither is there effective treatment available.

The first studies characterized stimulation tests (multiple rapid swallowing, bread swallow and swallow with abdominal compression) in healthy volunteers. Subsequent studies assessed the therapeutic effect of Azithromycin in patients with IOM and the possible predictive factors for prokinetic therapy.

I performed HRM in the semi-recumbent position in order to eliminate the effect of gravity on oesophageal motility. However, most dysphagia or reflux symptoms are perceived in the upright position. It may be useful to perform HRM in an upright position to establish normal values in more physiological conditions.

Weak proximal motility is associated with presentation of the reflux symptoms. Patients with respiratory symptoms suspected of being secondary to oesophageal origin may benefit from the study of proximal oesophageal motility.

Azithromycin as a pilot study demonstrated indication of significant prokinetic effects on normalising IOM in a subgroup of patients with IOM. The treatment effects of AZI on oesophageal motility in the longer-term, the effect on symptoms and reflux parameters require further studies.

Our preliminary results suggested that provocative tests such as multiple rapid swallowing and/or swallows with abdominal compression may assess oesophageal peristaltic reserve in the oesophageal body. This might have clinical value in the evaluation of patients with IOM to predict the response to prokinetic therapy. Further research is warranted to reproduce our results, and to determine if prokinetic therapy needs to be tailored to the response to MRS and swallow with abdominal compression.

It would be worthwhile investigating the effect of newer prokinetic medications such as Prucalopride (a serotonin 5-HT₄ agonist)(334), Mirtazapine (a noradrenergic and specific serotonergic antidepressant) (335) and Relamorelin (a ghrelin agonist) (336) on oesophageal motility in order to provide further treatment options for patients with IOM.

Moreover, other stimulation tests such as edrophonium and bethanechol challenge tests may also play important role in further phenotyping the IOM patients and help with identifying and targeting the patients who have the potential to respond to prokinetic therapy.

The neuromuscular reserve in Parkinson's disease and the elderly associated with IOM has not been studied and further investigations in this area could be invaluable to overcome dysphagia in these groups of patients.

The role of hypomotility in refractory GORD has long been a matter for debate as to whether it is the consequence or the cause of GORD. It could be extremely valuable to study the effect of prokinetic therapy with the new targeting approach discussed in this PhD thesis on this group of patients. Similarly, Barrett's oesophagus is associated with hypomotility (337) and it is not known whether it is reversible after endoscopic ablation of the affected area of oesophagus and whether this can be predicted by stimulation tests.

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