Post-Surgical Hypoparathyroidism (PoSH): Causes, consequences and novel preventative techniques

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A thesis submitted for the degree of Doctor of Philosophy

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18th February, 2022
To my wonderful daughters, Eleanor and Isabelle
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Abstract

Post-surgical hypoparathyroidism (PoSH) is a significant cause of hypocalcaemia following thyroid and parathyroid surgery. Hypocalcaemia can be temporary or long-term and reported incidences vary greatly in the literature. PoSH is associated with significant morbidity and requires ongoing treatment. Several studies have found that patients with PoSH experience a lower quality of life.

Management of post-thyroidectomy hypocalcaemia is guided by recommendations from the British Association of Endocrine and Thyroid Surgeons UK. However, the management of post-parathyroidectomy hypocalcaemia has little evidence to support guidance. This thesis evaluates the epidemiology of post-surgical hypoparathyroidism after parathyroid surgery and its current management in Sheffield Teaching Hospitals NHS Foundation Trust. This showed that low post-operative parathyroid hormone levels, rather than calcium itself, was a good indicator for the development of PoSH.

Although several studies report on quality of life in post-surgical hypoparathyroidism they are either not matched to appropriate controls or are small studies and recommendations from a systematic review was that further studies considering co-morbidities and aetiology should be performed. For this thesis, a cross-sectional observational study of four hundred and thirty-nine participants including matched controls showed that patients with PoSH reported significantly more fatigue and loss of energy.

Intra-operative preservation of parathyroid glands is key to the prevention of PoSH, and especially long-term PoSH. Parathyroid gland identification has largely relied on surgical technique and experience. Several novel technologies are being investigated to aid the intra-operative identification of the glands and assessment of viability. Two of these technologies are investigated in this thesis – near infrared fluorescence and electrical impedance spectroscopy. Two Phase I clinical trials were conducted to evaluate the feasibility of these technologies in thyroid and parathyroid surgery. Both technologies showed potential but near infrared fluorescence was the most promising with an important and interesting observation of parathyroid auto-fluorescence.
Publications, presentations and awards granted

Publications

Use of electrical impedance spectroscopy for intraoperative tissue differentiation during thyroid and parathyroid surgery

SL Hillary, BH Brown, NJ Brown, SP Balasubramanian

Use of methylene blue and near infrared fluorescence in thyroid and parathyroid surgery

Hillary, S.L., Guillermet, S., Brown, N.J. et al.

Oral Presentations

Quality of life in post-surgical hypoparathyroidism (PoSH)

SL Hillary, E Glenister, JE Chooi, K Farnell, J Grey, J Wadsley, JD Newell-Price, NJ Brown, SP Balasubramanian
BAETS, BJS Prize session, Leeds, 7-8 October 2021

Use of electrical impedance spectroscopy for intraoperative tissue differentiation during thyroid and parathyroid surgery

SL Hillary, BH Brown, NJ Brown, SP Balasubramanian
WCS, Krakow 11-15th August 2019

Exogenous contrast for parathyroid detection

SL Hillary
Invited speaker, 1st International Symposium on Parathyroid Identification and Parathyroid Angiography with Fluorescence and other techniques
Geneva, Switzerland, February 28th – March 1st 2019

Differentiation of tissue types in thyroid and parathyroid surgery using electrical impedance spectroscopy

S Hillary, B Brown, P Highfield, S Balasubramanian
BAETs, BJS Prize Session, Belfast 10th-13th October 2017
Abstract published in European Journal of Surgical Oncology
Intraoperative localisation of a pancreatic neuroendocrine tumour using real-time near infrared fluorescence with methylene blue

S Hillary, A Munir, S Guillermet, A Al-Mukhtar, A Hopper, SP Balasubramanian
Joint Meeting CAEKS – BAETS, BJS Prize Session, Berlin 10th – 12th November 2016
Abstract published in Langenbeck’s Archives of Surgery

Poster presentations

Intraoperative near infrared fluorescent imaging of methylene blue in parathyroid identification – a phase Ib human study

S Hillary, B Ellison-Handley, E Collins, P Truran, C Lim, B Harrison, M Berthoud, S Guillermet, N Brown, S Balasubramanian

Awards

IAES Travel Award – WCS Krakow, Poland
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I am thankful to the patients and participants who have contributed to the studies. Without their selfless contributions we would not be making advances in technology that we hope will improve outcomes to future patients. I would also like to thank the staff in the Operating Department who have supported the studies. Particular thanks to Mr Barney Harrison, Miss Beverly Lim, Miss Emma Collins, Mr Peter Truran, Dr Mireille Berthoud and Dr Martin Feast. I also owe a debt of gratitude to Branwen Ellison-Handley who supported me through my first clinical trial.

I will be forever indebted to my wonderful parents, Keith and Marnie, who have provided unwavering support, interest and childcare.

Finally, I dedicate this thesis to my husband Chris and my daughters Eleanor and Isabelle. There have been several life-changing moments throughout the last seven years I have worked on this research and, throughout it all, they have provided encouragement and purpose.

Sarah Louise Hillary
Sheffield, UK
2020
# Abbreviations

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<tr>
<td>PoSH</td>
<td>Post-surgical hypoparathyroidism</td>
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<tr>
<td>5-ALA</td>
<td>5-aminolevulinic acid</td>
</tr>
<tr>
<td>AMEND</td>
<td>Association of Multiple Endocrine Neoplasia Disorders</td>
</tr>
<tr>
<td>BAETS</td>
<td>British Association of Endocrine and Thyroid Surgeons UK</td>
</tr>
<tr>
<td>BNE</td>
<td>Bilateral neck exploration</td>
</tr>
<tr>
<td>BTCT</td>
<td>Butterfly Thyroid Cancer Trust</td>
</tr>
<tr>
<td>CIN</td>
<td>Cervical intra-epithelial neoplasia</td>
</tr>
<tr>
<td>CND</td>
<td>Central neck dissection</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
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<tr>
<td>eGFR</td>
<td>Estimated glomerular filtration rate</td>
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<tr>
<td>EI</td>
<td>Electrical impedance</td>
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<tr>
<td>EIS</td>
<td>Electrical impedance spectroscopy</td>
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<tr>
<td>EMA</td>
<td>European Medicines Agency</td>
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<tr>
<td>EoSRES</td>
<td>East of Scotland Research Ethics Service</td>
</tr>
<tr>
<td>FDA</td>
<td>Federal Drug Administration</td>
</tr>
<tr>
<td>FNA</td>
<td>Fine needle aspiration</td>
</tr>
<tr>
<td>GCP</td>
<td>Good clinical practice</td>
</tr>
<tr>
<td>GP</td>
<td>General Practitioner</td>
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<tr>
<td>HBS</td>
<td>Hungry bone syndrome</td>
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<tr>
<td>HcSS</td>
<td>Hypocalcaemia symptom score</td>
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<td>HPT</td>
<td>Hypoparathyroidism</td>
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<tr>
<td>ICG</td>
<td>Indocyanine green</td>
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<td>IMP</td>
<td>Investigational medicinal product</td>
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<tr>
<td>IOPTH</td>
<td>Intra-operative parathyroid hormone assay</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous</td>
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<tr>
<td>LED</td>
<td>Light emitting diode</td>
</tr>
<tr>
<td>MB</td>
<td>Methylene blue</td>
</tr>
<tr>
<td>MEN</td>
<td>Multiple endocrine neoplasia</td>
</tr>
<tr>
<td>MHRA</td>
<td>Medicines and Healthcare Regulatory Agency</td>
</tr>
<tr>
<td>MOS</td>
<td>Medical outcomes study</td>
</tr>
<tr>
<td>NHS</td>
<td>National Health Service</td>
</tr>
<tr>
<td>NICE</td>
<td>National Institute for Clinical and Health Excellence</td>
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<tr>
<td>NIR</td>
<td>Near infrared</td>
</tr>
<tr>
<td>NIRF</td>
<td>Near infrared fluorescence</td>
</tr>
<tr>
<td>NP</td>
<td>normal parathyroid</td>
</tr>
<tr>
<td>NV</td>
<td>Normalised value</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
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<tr>
<td>PA</td>
<td>Parathyroid auto-transplant</td>
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<tr>
<td>PG</td>
<td>Parathyroid gland</td>
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<tr>
<td>PHPT</td>
<td>Primary hyperparathyroidism</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>pNET</td>
<td>Pancreatic neuroendocrine tumour</td>
</tr>
<tr>
<td>POD1</td>
<td>Post-operative day 1</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>QA</td>
<td>Quality assurance</td>
</tr>
<tr>
<td>QC</td>
<td>Quality control</td>
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<tr>
<td>QOL</td>
<td>Quality of life</td>
</tr>
<tr>
<td>REC</td>
<td>Regional Research Ethics Committee</td>
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<tr>
<td>RLN</td>
<td>Recurrent laryngeal nerve</td>
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<td>ROC</td>
<td>Receiver operating characteristic</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
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<td>SF-36</td>
<td>RAND 36 Item Health Survey</td>
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<tr>
<td>SHPT</td>
<td>Secondary hyperparathyroidism</td>
</tr>
<tr>
<td>SID</td>
<td>Study identification</td>
</tr>
<tr>
<td>SM</td>
<td>Spectrum mean</td>
</tr>
<tr>
<td>SPECT</td>
<td>single photon emission computerised tomography</td>
</tr>
<tr>
<td>SSRI</td>
<td>Serotonin re-uptake inhibitors</td>
</tr>
<tr>
<td>STH</td>
<td>Sheffield Teaching Hospitals NHS Foundation Trust</td>
</tr>
<tr>
<td>STHNFT</td>
<td>Sheffield Teaching Hospitals NHS Foundation Trust</td>
</tr>
<tr>
<td>STM</td>
<td>spectrum template match</td>
</tr>
<tr>
<td>THPT</td>
<td>Tertiary hyperparathyroidism</td>
</tr>
<tr>
<td>TM</td>
<td>template match</td>
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<td>TP</td>
<td>Targeted parathyroidectomy</td>
</tr>
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<td>TSH</td>
<td>Thyroid stimulating hormone</td>
</tr>
<tr>
<td>TV</td>
<td>template value</td>
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<tr>
<td>UKRETS</td>
<td>United Kingdom Registry of Endocrine and Thyroid Surgeons</td>
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<tr>
<td>UNE</td>
<td>Unilateral neck exploration</td>
</tr>
<tr>
<td>V</td>
<td>Value</td>
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<td>World Health Organisation</td>
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Chapter 1: Introduction
Surgery to remove diseased glands in the central compartment of the neck is common with around 18,000 operations on the thyroid and parathyroid glands recorded by Health Episode Statistics for England in 2016-2017 (Statistics 2017, Sitges-Serra, Lorente-Poch et al. 2018). Patients consent to surgery with the understanding that there may be complications but expect that the operation will make them better or reduce the risk of future complications, not worse. One of the recognised complications of thyroid and parathyroid surgery is hypoparathyroidism, where the function of the parathyroid glands is impaired, this manifests itself as hypocalcaemia (low serum calcium). This can have significant implications to the patient’s wellbeing.

Post-surgical hypoparathyroidism (PoSH) is the term given to the condition of impaired parathyroid function as a consequence of surgery. Accurately identifying the glands intra-operatively ensures they can be preserved or, should the vascular supply to the gland be disrupted, they can be auto-transplanted. This is when the parathyroid gland is re-implanted into a muscle to regain a blood supply and maintain its function, which has been shown to reduce permanent PoSH (Lo 2002). It can be difficult for surgeons to be certain that they have correctly identified the gland but also the assessment of its viability is subjective. There is scope for intra-operative technology that aid parathyroid identification to reduce the incidence of PoSH thereby improving surgical outcomes, patients’ health and wellbeing and reducing costs to the NHS by reducing the follow up and treatment required following surgery.

This thesis will evaluate novel technologies that may aid intra-operative identification and preservation of parathyroid glands and their function. The two technologies focused on in this thesis are near infrared fluorescence imaging and electrical impedance spectroscopy. Early phase clinical trials tested these two novel technologies in human subjects following work in animal models.

The British Association of Endocrine and Thyroid Surgeons recently reported a 6.5% rate of permanent hypocalcaemia following thyroidectomy (Chadwick D 2017). Patients with PoSH have longer hospital stays, increased outpatient appointments and investigations, increasing the workload of the staff and placing a financial burden
on an already stretched health service. Patients are commenced on treatment which must be taken daily incurring prescription charges. Patients can become symptomatic of hypocalcaemia if treatment is sub therapeutic or suffer sequelae from long-term treatment, all of which can have significant impact on their quality of life. There is an understanding amongst clinicians that patients with PoSH report worsened overall well-being when compared to other groups of patients with long-term conditions. The underlying cause for this is not well understood but some recent investigations have tried to uncover what effects PoSH has on quality of life (McIntyre, Di Marco et al. 2017).

In this thesis, the impact PoSH has on patients’ quality of life has been examined in detail through an observational study of patients who have previously had neck surgery comparing patients with and without PoSH. The burden of PoSH after parathyroid surgery has been qualified through an audit of patients with hypocalcaemia following parathyroid surgery. It investigates their management and treatment and compares them to national standards and identifies potential areas for improvement.

1.1 Parathyroid structure and function

There are usually four parathyroid glands located in the neck. They are in close association with the thyroid gland (Figure 1.1). The thyroid is a butterfly shaped gland with two lobes connected by the central isthmus and is found in the lower part of the neck, anterior to the top three tracheal rings. The parathyroid glands are relatively much smaller in size; normal glands being compared to the size of a grain of rice, weighing around 40 mg, are light brown to reddish-brown in colour and have a smooth capsule. The glands can be difficult to distinguish from other common structures in the central neck compartment such as thyroid nodules, adipose tissue or lymph nodes because they have a similar size, shape, colour and consistency.

The parathyroid glands usually sit in pairs on either side of the thyroid (Akerstrom, Malmaeus et al. 1984). The superior glands are generally more constant in location and can be identified posterior-lateral to the superior pole of the thyroid at the cricothyroid junction (Figure 1.2). They originate from the fourth pharyngeal pouch
and have a close association with the thyroid gland and only migrate a short distance during neck development (Figure 1.3). The inferior glands, however, are more variable in their position but usually lie close to the inferior pole of the thyroid or within the thyro-thymic tract. The reason for this can be traced back to embryological development. Both the thymus and inferior parathyroid glands develop from the third pharyngeal pouch and as the thymus descends towards the mediastinum, the parathyroid glands also descend with it. The parathyroid glands normally separate from the thymus and are therefore usually found towards the lower pole of the thyroid. If this separation occurs late or fails to occur, the inferior parathyroid may be adjacent to or contained within the thymus. Ectopic glands may also be found anywhere in the path of migration of the thymus from mandible to pericardium, including within the carotid sheath. Parathyroid glands can occasionally be contained within the thymus.

Figure 1.1  The central neck compartment

The central neck compartment is bordered by the hyoid bone superiorly, carotid arteries laterally and the sternal notch inferiorly. The lymph nodes within in the neck are divided into anatomical levels. The central neck includes level VI and level VII nodes. Within the central neck compartment the thyroid is located anterior to the top three tracheal rings. The parathyroid glands are usually found behind the thyroid gland. (Shirley, Jones et al. 2017)
Supernumerary glands can also occur and are usually located near the inferior glands, within the thymus or in the mediastinum.

Superior and inferior glands occasionally lie close together but can be anatomically differentiated by their relation to the recurrent laryngeal nerve (Figure 1.2). Superior glands are usually deeper or posterior to the recurrent laryngeal nerve and the inferior glands anterior to it (Lappas, Noussios et al. 2012).

As illustrated in Figure 1.3, the parathyroid glands have variable locations and therefore have a variable blood supply, often dependent on their position (Johansson, Ander et al. 1994). Frequently both the inferior and superior glands receive vascular supply from the inferior thyroid artery. However, in many patients the superior parathyroid can also be supplied by a posterior branch of the superior thyroid artery (Nobori, Saiki et al. 1994). If the inferior parathyroid gland is low in the

**Figure 1.2 Relationship of the parathyroid glands to the recurrent laryngeal nerve**

The relationship of the parathyroid glands to the recurrent laryngeal nerve. The superior gland is found posterior or deep to the nerve whilst the inferior gland is usually in front or anteriorly to it. Although superior glands are usually above the inferior thyroid artery, enlarged glands can prolapse down and be located below it. (Hillary and Balasubramanian 2017)
mediastinum the blood supply may come from the thymic branch of the internal thoracic artery or a branch directly from the aortic arch.

Parathyroid glands are responsible for regulation of serum calcium. Calcium is an important ion for normal function of the heart, nervous system, muscle contraction, kidneys and bone. It is also an essential co-factor for clotting and various pathways of metabolism. Parathyroid glands produce parathyroid hormone (PTH) and are stimulated in the presence of low blood calcium levels (hypocalcaemia) (Figure 1.4) (Liyanarachchi and Debono 2017). PTH influences multiple organs to increase serum calcium levels. Direct effects include increased reabsorption in renal tubules and mobilisation of calcium from bone. Indirect effects include increased absorption of

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**Figure 1.3  Embryological development and descent of the parathyroid glands**

*Embryological development and descent of the parathyroid glands from the third and fourth branchial pouches (Hillary and Balasubramanian 2017). Parathyroid glands can be located at any point on the line of descent and therefore have a variable location. Intra-operative localisation of the glands can be difficult due to their variable location and uncertainty can be had as they are similar in appearance to fat, lymph nodes and thyroid nodules.*
calcium from the gut via activation of vitamin D. A high serum calcium level inhibits synthesis and release of PTH. This feedback system ensures homeostasis of blood calcium levels in individuals with normal parathyroid function.

**Figure 1.4  Calcium homeostasis**

*PTH production is triggered by low serum calcium which exerts its effects on various systems to increase serum calcium. If there is overproduction of PTH due to adenoma or hyperplasia, then these systems continue to increase calcium above a normal level. If the parathyroid glands are damaged, then PTH is not produced at the levels required to maintain a normal calcium level.*
1.2 Neck surgery in the central compartment

Surgery is performed in the central compartment of the neck for diseases of the thyroid, parathyroid glands, larynx and/or pharynx. Thyroid and parathyroid surgery are common operations; around 18,000 procedures being performed each year in England alone (Statistics 2017). These procedures are carried out by both endocrine and head and neck surgeons for a number of benign and malignant conditions. Surgical procedures on the larynx and pharynx are common with over 42,000 performed in 2016-2017 (Statistics 2017) but excision is far less common (700 laryngectomies or pharyngectomies performed in 2016-2017 (Statistics 2017)). Complications following thyroid and/or parathyroid surgery include bleeding, recurrent laryngeal nerve injury with potential for significant voice change and damage to the parathyroid glands causing hypocalcaemia (Chadwick 2012). Surgical technology is rapidly evolving and aimed at improving clinical outcomes and increasing patient safety. Endocrine surgery has seen many developments within recent years with the introduction of nerve stimulators (Barczynski, Konturek et al. 2012) and energy devices for dissection and sealing, to reduce the risk of complications arising from nerve injury or bleeding (Yao, Wang et al. 2009).

Identification and preservation of the parathyroid gland and its blood supply is integral to maintaining calcium homeostasis and reducing the incidence of post-surgical hypoparathyroidism (PoSH) (Thomusch, Machens et al. 2003). Currently, parathyroid identification and preservation relies on surgical technique and expertise. However, even in high volume centres with experienced surgeons, PoSH is a common problem (Chadwick 2012).

1.2.1 Thyroid surgery

Operations on the thyroid gland are usually performed for one of several reasons: hyperthyroidism, goitre and suspected or confirmed thyroid cancer (Vanderpump 2011).
The thyroid gland produces thyroxine under the control of thyroid stimulating hormone (TSH) released by the anterior pituitary gland. Thyroid dysfunction includes over activity (hyperthyroidism) or under activity (hypothyroidism).

Primary hyperthyroidism can be due to Graves’ disease, toxic multinodular goitre or toxic adenoma (Rowland and Shore 2017). Several treatment options including anti-thyroid drug treatment, radioiodine ablation and surgery are available and have specific roles in the management of these conditions. Surgical treatment is a reasonable therapeutic option with high efficacy, and is particularly appropriate when medical treatment or radioiodine ablation is poorly tolerated or contraindicated (Vaidya and Pearce 2014).

A goitre is enlargement of the thyroid and is usually benign and most patients have normal thyroid function (euthyroid). The commonest indication for an operation in these patients is the presence of significant compression of the trachea and/or oesophagus. Thyroid cancer is rare, (incidence 7.9 per 100,00 in females and 3.4 per 100,00 in males (Statistics 2014)) but suspected or confirmed cancer is another common reason for thyroidectomy.

The thyroid can be resected wholly or partially, dependent on pathology. If the problem is isolated to a single lobe such as a dominant or indeterminate nodule, a hemi-thyroidectomy (affected lobe with the isthmus) is performed (Rowland and Shore 2017). When the pathology affects the entire gland, such as in Graves’ disease, multinodular goitre or cancer; a near-total or total thyroidectomy is performed.

Thyroid cancer, particularly papillary thyroid cancer, can spread to the local lymph nodes. The lymph nodes within the neck are divided anatomically into seven levels. Thyroid cancer usually spreads to nodes found within level VI and VII first (Figure 1.1), which make up the ‘central neck compartment’. Level VI is bounded by the hyoid bone superiorly, the sternal notch inferiorly and the medial border of the carotid artery laterally. Level VII nodes are found below this, between the sternal notch and the innominate vein. These lymph nodes may also be called the pre-laryngeal, pre-tracheal and para-tracheal nodes. Central neck dissection (CND) is when the lymph nodes within this compartment with surrounding fatty tissue and fascia are removed.
due to proven or suspected metastatic spread. This compartment may be divided into right and left (either side of the midline) and either ipsilateral or bilateral CND can be performed. Within this level both the recurrent laryngeal nerve (RLN) and the parathyroid glands are found. CND is associated with a higher rate of RLN injury and hypoparathyroidism. The differences in complications of patients undergoing thyroidectomy with bilateral CND was compared to thyroidectomy with ipsilateral CND and no CND in an Italian series of 1087 patients (Giordano, Valcavi et al. 2012). The study identified a statistically higher rate of permanent hypoparathyroidism in the bilateral CND group compared to the other groups (bilateral 16%, ipsilateral 7% and no CND 6.3%). Although there was no significant increase in permanent RLN injury, the bilateral group showed an increased injury rate (bilateral 2.3%, ipsilateral 0.5%, no CND 1%) (Giordano, Valcavi et al. 2012). The 5th National Audit by the British Associations of Endocrine and Thyroid Surgeons (BAETS) reported that over one third of patients who had a bilateral CND as part of a thyroidectomy developed early hypocalcaemia (Chadwick D 2017).

1.2.2 Parathyroid surgery

Parathyroid gland dysfunction includes hyperparathyroidism or hypoparathyroidism; the former being a much more common problem. This can be classified into three broad categories: primary hyperparathyroidism (PHPT), secondary hyperparathyroidism (SHPT) and tertiary hyperparathyroidism (THPT). The secondary and tertiary forms are often seen in association with end stage renal disease and are referred to as renal hyperparathyroidism.

In PHPT, there is autonomous and excessive synthesis and secretion of PTH independent of serum calcium levels. This is usually due to a benign tumour of one or more parathyroid glands. The raised PTH level results in hypercalcaemia and these are associated with consequences including osteoporosis, renal stones, renal impairment and a number of symptoms (Kebebew and Clark 1998). In the majority of patients, one gland is affected but around 15% of patients can have two to four glands involved (Haciyanli, Lal et al. 2003, Bergenfelz, Kanngiesser et al. 2005). Surgery aims to remove the affected gland(s), therefore reducing PTH and correcting
hypercalcaemia. Abnormal glands can often be identified pre-operatively with imaging techniques including high-frequency ultrasound and sestamibi scintigraphy scans (Chien and Jacene 2010). This facilitates a targeted operation in patients with single gland disease (Uruno and Kebebew 2006). However, these imaging techniques do not always reveal the affected gland (Khorasani and Mohammadi 2014) and are not useful in multi gland disease; in the latter up to three and half glands may be removed at surgery.

Patients with kidney failure have derangement in several metabolic parameters (including high phosphate levels, low calcium and vitamin D levels and renal resistance to PTH) that results in over stimulation, synthesis and secretion of PTH (Pallan, Rahman et al. 2012). Unlike patients with PHPT, these patients often have a normal or low serum calcium. In some instances where the parathyroid glands become autonomous (called tertiary hyperparathyroidism), serum calcium is high. Surgery in these instances involves either a subtotal or total parathyroidectomy with auto-transplantation.

1.2.3 Larynx and pharynx surgery

Total laryngectomy is predominantly performed for advanced laryngeal cancers or when other less radical procedures or treatments have failed (Bethesda 2015). Near-total laryngectomy and supracricoid laryngectomy are other surgical options for small tumours and allow speech by reconstruction of the vocal cords when the tumour is small and suitably positioned. Depending on the extent of the tumour, pharyngectomy may also be performed. During total or near-total laryngectomy, part or all of the thyroid may be excised as part of the specimen.

1.3 Adverse effects of surgery on the central neck

1.3.1 Hypocalcaemia and hypoparathyroidism

Hypocalcaemia may occur after any of the above types of operations. Post-operative hypocalcaemia refers to an acute drop in serum calcium immediately after surgery. It can manifest itself with a number of symptoms including circumoral paraesthesia,
tetany, carpopedal spasm, laryngospasm, electrocardiogram (ECG) changes and even seizures or cardiac arrest in severe, untreated cases (Cooper and Gittoes 2008). It is transient in most cases but can be protracted or even permanent in a small population. Hypocalcaemia occurs for two main reasons. Patients undergoing parathyroidectomy for severe PHPT can be susceptible to ‘hungry bone syndrome’. In these cases where high PTH levels have caused high bone turnover, the drop in PTH to physiological levels after parathyroidectomy allows bone remodelling using serum calcium reserve (Witteveen, van Thiel et al. 2013). The more common cause of hypocalcaemia however is hypoparathyroidism where the parathyroid glands are damaged and do not produce adequate amounts of PTH to maintain calcium homeostasis. Patients consenting for thyroid surgery are advised that the risk of requiring long term treatment for hypocalcaemia is 5-7% and is increased to 5-10% in patients with hyperactive thyroid disease or thyroid cancer (Tolley 2019). The need for calcium and/or vitamin D supplements to maintain normocalcaemia in the long term is used as a surrogate marker of post-surgical hypoparathyroidism and is an important outcome of surgery. It is one of the outcomes specifically audited in the United Kingdom Registry of Endocrine and Thyroid Surgeons (UKRETS) database. In the UK the fourth national audit by BAETS reported a 25% incidence of transient hypocalcaemia and a 12% incidence of long term or permanent hypocalcaemia (Chadwick 2012). The fifth national audit published in 2017 stated a rate of 3.5% for long term hypocalcaemia following first time thyroid surgery (Chadwick D 2017). This reduction in long term hypocalcaemia may be attributed either to more consistent definition of this complication or improved surgical performance (Chadwick D 2017). The data in the national audit is based on self-reported outcomes by members of BAETS and not externally validated. Not all surgeons performing these procedures are members of the association and therefore a significant proportion of UK surgeons do not contribute to the audit. It is estimated that thyroidectomy data in the UKRETS database is likely to represent 40% of the total operations performed annually in the UK. In addition, members contributing to the database do not have a 100% completion rate, as there is missing data on outcomes for a significant number of patients. Data on post-operative and late hypocalcaemia (i.e., six months after surgery) has one of the highest rates of missing data. Missing data rates rise from 8%
for post-operative hypocalcaemia to 19% for late hypocalcaemia (Chadwick, Kinsman et al. 2017). Although the most significant and long term cause of post-operative hypocalcaemia is due to hypoparathyroidism (PoSH), in some instances transient hypocalcaemia could be aggravated by or be solely due to other factors such as hungry bone syndrome and vitamin D deficiency (Cooper and Gittoes 2008).

Patients in most centres stay overnight following thyroid or parathyroid surgery allowing testing of serum calcium the following day. Patients usually recover quickly, and post-operative pain is usually well tolerated allowing early discharge from hospital. However, hypocalcaemia can significantly extend hospital stay. A recent nationwide study showed that hypocalcaemia after thyroidectomy extended stay by 1.47 days (Baldassarre, Chang et al. 2012) thus increasing healthcare costs and decreasing patient satisfaction. Mild hypocalcaemia (asymptomatic patient, >1.9 mmol/L) can be managed with oral calcium and/or active vitamin D supplements and repeat biochemical measurements within a week. Parent (inactive) Vitamin D supplementation should also be started in those found to be vitamin D deficient. Severe hypocalcaemia (<1.9 mmol/L and/or symptomatic at any level below the reference range) is a medical emergency. Patients may require intravenous infusions with IV calcium gluconate followed by oral replacement therapies as required. Patients are then followed up and tested a week after discharge and then if blood levels are satisfactory, follow up is at one, three and six months (Cooper and Gittoes 2008, Society for Endocrinology's Clinical Committee 2013).

1.3.2 Post-surgical hypoparathyroidism

Post-operative hypocalcaemia is primarily caused by a reduction in circulating PTH levels. This is in turn due to parathyroid gland damage at surgery. Normal parathyroid tissue, even if it has been suppressed by an overactive gland (as in primary hyperparathyroidism), recovers quickly and calcium control is rapidly regained (Youngwirth, Benavidez et al. 2010). Parathyroid gland damage can be due to direct injury, devascularisation or inadvertent excision during the surgical procedure (Page and Strunski 2007, Edafe, Antakia et al. 2014). Inadvertent excision of a parathyroid gland is not uncommon during thyroidectomy and occurs when the parathyroid
glands are not positively identified as part of the procedure (Sakorafas, Stafyla et al. 2005). Prevalence of post-surgical hypoparathyroidism was underestimated until around the year 2000 until multicentre and registry studies were published (Sitges-Serra, Lorente-Poch et al. 2018). A retrospective review at a centre in Ireland looked at inadvertent parathyroidectomy during thyroidectomy (McGoldrick, Majeed et al. 2017). Over two years, 230 thyroidectomies were performed (147 hemithyroidectomy, 83 total thyroidectomy) and included 13 central neck dissections. 40 patients (17.3%) had inadvertent parathyroidectomy. They noted that malignancy and central neck dissection increased the risk of inadvertent excision (McGoldrick, Majeed et al. 2017).

A reduction in circulating PTH prevents the breakdown of bone, reducing calcium release and promotes the formation of new bone, using up blood calcium supplies. Low PTH levels also increases renal excretion and decreases gut reabsorption (Cartwright and Anastasopoulou 2021).

There is no universal consensus on terminology surrounding post-operative hypoparathyroidism (Ritter, Elfenbein et al. 2015, Edafe and Balasubramanian 2017). There is variation in the suggested cut-off levels and differences in whether the need for treatment should be considered in the definition. There is also controversy on the threshold between “temporary” and “permanent” hypoparathyroidism; terms often referred to in many manuscripts. BAETS defines hypocalcaemia as “early” or “late” (Chadwick 2017). Early hypocalcaemia is defined as a corrected serum calcium of <2.10 mmol/L on the first day following surgery. Late hypocalcaemia is defined as the condition that requires continued calcium and/or vitamin D supplementation to maintain normocalcaemia at six months following surgery (Chadwick 2017). These patients cannot be considered to have permanent hypoparathyroidism as their parathyroid function may recover in the future and supplementation may be able to be stopped. The BAETS definitions do not take into consideration whether the patient is symptomatic. Some studies will only define a patient as developing hypoparathyroidism if their serum calcium is below the reference range and the patient is symptomatic or requiring treatment (Lorente-Poch, Sancho et al. 2015, Stack, Bimston et al. 2015). Some surgeons start prophylactic calcium supplements
post-operatively to prevent hypocalcaemia thus skewing post-operative hypocalcaemia rates in the literature further (Terris, Snyder et al. 2013, Antakia, Edafe et al. 2014, Edafe, Mech et al. 2019). Due to these inconsistencies in definitions and practice, the incidence of post thyroidectomy hypoparathyroidism varies greatly in the literature available (Ritter, Elfenbein et al. 2015, Htun, Edmiston et al. 2018). Another cause for the variation in reported incidence is the inclusion of procedures not involving bilateral thyroid surgery, which have a much lower incidence of hypoparathyroidism as one pair of parathyroid glands remain untouched (Del Rio, Rossini et al. 2019). Published studies report that between 19%-38% of patients develop hypocalcaemia (Edafe, Antakia et al. 2014). Patients requiring treatment one month after surgery have a 25% chance of developing late hypocalcaemia (Lorente-Poch, Sancho et al. 2015). Patients with persistent late hypocalcaemia require lifelong supplements and can develop significant morbidity including renal stones, nephrocalcinosis and soft tissue calcification (Khan, Waguespack et al. 2011, Mitchell, Regan et al. 2012). In addition, there appears to be a significant impact on quality of life (Cho, Moalem et al. 2014), which will be discussed in greater detail later in this chapter.

There are limited studies on the prevalence of hypoparathyroidism. A recent population based study in Scotland reviewed the epidemiology of chronic hypoparathyroidism and reported that PoSH is the commonest cause of hypoparathyroidism (Vadiveloo, Donnan et al. 2018). The study identified 18,955 patients with hypocalcaemia (at least three serum albumin-corrected calcium measurements taken one month apart as an outpatient that were below the reference range) between 1988 and 2015. A total of 280 patient had 'chronic hypoparathyroidism', of which, 116 were attributed to PoSH (Vadiveloo, Donnan et al. 2018, Vadiveloo, Donnan et al. 2019). The study concluded that the prevalence of PoSH was 23 per 100,000 in 2015 (Vadiveloo, Donnan et al. 2018). A Danish population-based study (Underbjerg, Sikjaer et al. 2013) identified 980 patients with PoSH, defined as hypocalcaemia with inappropriately low PTH following neck surgery requiring treatment for more than 6 months, over a similar time period (1988-2012) from the Danish National Patient Registry and prescription database. The prevalence of long term PoSH was estimated to be 22 per 100,000 (Underbjerg, Sikjaer et al.)
An American study (Powers, Joy et al. 2013) used a large insurance claims database to estimate the prevalence of hypoparathyroidism, identifying all cases over a 12-month period (October 2007-September 2008) and extrapolated the numbers to the insured US population. 58793 patients had long term (not resolved within 6 months) hypoparathyroidism; a prevalence of long term PoSH of 23 per 100,000 (Powers, Joy et al. 2013). Using the Norwegian Electronic Hospital Record the prevalence of long term PoSH, defined as low serum calcium and inappropriately low PTH requiring treatment for more than 1 year was estimated to be 6.4 per 100,000 (Astor, Lovas et al. 2016). Across these four patient populations the prevalence of PoSH is comparable (except for the Norwegian cohort which is relatively low); although the definitions of the condition vary between the studies.

1.3.3 Predictive and preventative factors for post-surgical hypoparathyroidism

In thyroid surgery, a number of predictive factors for PoSH have been reported (Edafe, Antakia et al. 2014). Biochemical predictors of transient hypocalcaemia include levels of pre-operative calcium, peri-operative PTH, pre-operative 25-hydroxyvitamin D and post-operative magnesium. Clinical predictors include surgery for recurrent goitre and re-operation for bleeding. A calcium level lower than 1.88mmol/l at 24h after surgery, identification of fewer than two parathyroid glands at surgery, re-operation for bleeding, Graves’ disease and heavier thyroid specimens have been identified as independent predictors of permanent hypocalcaemia (Edafe, Antakia et al. 2014). Factors associated with transient hypocalcaemia are inadvertent parathyroid gland excision, parathyroid gland auto-transplantation, Graves’ disease and female sex (Edafe, Antakia et al. 2014). Some preventative measures, such as different surgical techniques and prophylactic supplementation, have been reported to reduce the risk of hypoparathyroidism (Edafe, Mech et al. 2019). However, a recent review showed that post-operative calcium and vitamin D supplementation and bilateral subtotal thyroidectomy effectively reduced transient hypocalcaemia (Antakia, Edafe et al. 2015). Meticulous dissection of the parathyroid gland and preservation of its blood supply reduces the risk of developing hypocalcaemia (Lorente-Poch, Sancho et al. 2015) but for patients where there is injury,
devascularisation or inadvertent excision of the gland, parathyroid auto-
transplantation (PA) is a method of reducing permanent hypoparathyroidism
however associated with an increased incidence of transient hypocalcaemia, in
patients undergoing routine PA compared to selective cases (Lo 2002). Assessment
of devascularisation of parathyroid glands based on colour is subjective, dependent
on clinical judgement and experience (Lo 2002). A narrative review by Sitges-Serra et
al gave a historical account of normal parathyroid auto transplantation as part of a
total thyroidectomy, a technique proposed in the 1960s and 1970s (Sitges-Serra,
Lorente-Poch et al. 2018). The basis of this technique appears to be based on the
transplantation of hyperplastic tissue in renal or multi gland hyperparathyroidism
where abnormal parathyroid tissue is used. The authors stated that this was
“uncritically and enthusiastically” extrapolated to normal parathyroid transplantation
in thyroidectomy. There is not enough evidence to support selective, intentional auto
transplantation of a single, normal parathyroid gland in total thyroidectomy. The
authors suggested that this may, in fact, lead to hypoparathyroidism. The
recommended technique is to preserve normal parathyroid glands and their blood
supply in situ during thyroidectomy (Sitges-Serra, Lorente-Poch et al. 2018).

In parathyroid surgery, around 85% of patients diagnosed with primary
hyperparathyroidism have single gland disease (Pallan, Rahman et al. 2012).
Techniques have been developed to localise abnormal parathyroid glands pre-
operatively to facilitate targeted or unilateral surgery if possible. These usually
include a combination of imaging modalities such as high-frequency ultrasound and
sestamibi scintigraphy. The sensitivity of these scans is around 91% and 78%
respectively but sestamibi scintigraphy is less sensitive in patients with low pre-
operative PTH levels (Untch, Adam et al. 2011, Khorasani and Mohammadi 2014). Pre-
operative localisation of the gland provides an opportunity for a targeted
parathyroidectomy or a unilateral neck exploration (UNE) rather than bilateral neck
exploration (BNE). In the latter approach, attempts should be made to identify all
four glands; this results in significant dissection of the central neck compartment and
increased rates of transient hypocalcaemia (Bergenfelz, Kanngiesser et al. 2005,
These pre-operative techniques do not assist localisation of normal parathyroid glands, which need to be preserved to avoid PoSH. In patients undergoing parathyroid surgery, intra-operative localisation can be assisted by several techniques including frozen section, gamma probe, intra-operative parathyroid hormone assay and high dose intravenous Methylthioninium chloride (Methylene Blue, MB).

Post-surgical hypoparathyroidism is not limited to thyroid and parathyroid surgery alone. Hypoparathyroidism has also been reported in laryngopharyngeal surgery. One study of post laryngectomy patients showed a post-operative biochemical hypocalcaemia rate of 43% and 25% of patients had severe hypocalcaemia (Basheeth, O’Cathain et al. 2014).

1.4 Management of PoSH

Following diagnosis of hypoparathyroidism on biochemical testing patients are started on supplementation. Standard therapy in the UK is with calcium and/or vitamin D supplements (Bollerslev, Rejnmark et al. 2015). This treats the hypocalcaemia associated with PoSH rather than the hypoparathyroidism itself. PTH replacement therapies are currently unlicensed for use in the UK. In severe cases of acute hypocalcaemia, intravenous calcium supplementation may be required.

1.5 PoSH impact on patient wellbeing and quality of life

Hypoparathyroidism may present with symptoms but the symptoms of either under treatment and consequential hypocalcaemia or over treatment leading to hypercalcaemia can present as a wide spectrum of physical and psychological symptoms. Although this is a relatively rare condition, the impact on the population is significant enough to warrant a patient support charity. Parathyroid UK (www.parathyroiduk.org) is a charity based in the United Kingdom that supports patients with parathyroid disorders including PoSH. The patient information pages of the site include an extensive and varying list of symptoms attributed to both hyper and hypocalcaemia. The extent and array of symptoms is very variable between
individuals. It is not well understood why patients with hypoparathyroidism appear to have a lower quality of life than others with similar long-term conditions.

Hypoparathyroidism is associated with increased morbidity and mortality. Studies have shown that patients with post-surgical hypoparathyroidism have an increased risk of epilepsy and cataracts (Vadiveloo, Donnan et al. 2019). They have also been shown to have an increased incidence of renal calculi, chronic kidney disease and basal ganglia calcifications (Mitchell, Regan et al. 2012). Permanent hypoparathyroidism after total thyroidectomy has also been associated with increased mortality (Almquist, Ivarsson et al. 2018).

A systematic review by Buttner et al reviewed quality of life outcomes of patients with hypoparathyroidism receiving standard treatment. Five studies were included in the review and all studies used generic, validated questionnaires including the RAND 36 Item Health Survey (SF-36), World Health Organisation - Five Well Being Index (WHO-5), Symptom Checklist 90 Revised (SCL-90-R) and the Giessen Subjective Complaints List (GBB-24). All the studies included patients with PoSH. Two studies directly compared patients who had undergone surgery, with or without PoSH. They concluded that even when blood results are stable and within the normal ranges, patients report a lower quality of life than the normal population or matched controls. One theory for the cause of this, is that standard treatment includes supplementation of calcium with or without vitamin D rather than directly replacing PTH. The low PTH appears to have an effect on multiple body systems, reducing quality of life. PTH receptors have been discovered in the brain (Bago, Dimitrov et al. 2009), central nervous system (Balabanov, Tollner et al. 1984, Bago, Dimitrov et al. 2009) and muscle (Divieti, Inomata et al. 2001), which may explain its impact on mental health and physical manifestations. This review concluded that additional studies are needed to investigate this further and suggested that a disease specific questionnaire is required to identify important issues relevant to PoSH (Buttner, Musholt et al. 2017).

Shire, a global biotechnology company and developers of a parathyroid hormone injection to treat hypoparathyroidism, have conducted a market research survey of
patients living with chronic (treatment for longer than six months) hypoparathyroidism in association with Parathyroid UK (Shire 2017). 230 patients responded but patients were able to skip questions within the survey so not all questions were answered. 67.7% of respondents have PoSH. 38% of patients (n=211) reported a “significant impact” on their mental health. 34% of respondents (n=196) felt that hypoparathyroidism had a “severe impact” on their ability to maintain full time employment. The most common symptoms reported by the respondents were tiredness (n=215), tingling or numbness in the hands or feet (n=207), brain fog (n=198) and irritability (n=196). The survey also asked about quality of life and 76% of patients felt their hypoparathyroidism affected their ability to interact with their friends and family. 53% felt it affected their ability to drive a car and 67% reported that it affected their ability to go to the supermarket (Shire 2017). Although this research provides an interesting insight into the perceptions of patients with hypoparathyroidism it does not compare the responses to those of a control group and includes a third of patients who do not have PoSH.

To gain further insight into the quality of life of patients with post-surgical hypoparathyroidism we have performed a detailed assessment of symptoms and quality of life in different cohorts of patients with post-surgical hypoparathyroidism and compared these patients with similar cohorts of patients without hypoparathyroidism. This study took into account the aetiology of hypoparathyroidism, treatment and co-morbidities to analyse for possible predictive factors.

1.6 Novel technologies to improve intra-operative parathyroid identification and preservation

1.6.1 Emerging technologies

It is evident that PoSH is a significant complication of thyroid and parathyroid surgery with significant morbidity attached to it. Antakia et al reviewed the effectiveness of preventative measures in bilateral thyroid surgery in reducing post-operative hypocalcaemia (Antakia, Edafe et al. 2014). Preventative measures include
haemostatic techniques, extent of thyroidectomy, calcium and vitamin D supplementation, intra-operative parathyroid identification, truncal ligation of the inferior thyroid artery and use of loupes. The evidence was not conclusive as the studies varied widely in design and were of low quality. However, transient hypocalcaemia rates were significantly lower in the group where post-operative supplementation with calcium and vitamin D compared to calcium alone (odds ratio (OR)0.66; 95% confidence interval (CI) 0.45-0.98; p=0.05) or no supplements (OR 0.34; 95% CI 0.15-0.75; p=0.007). Transient hypocalcaemia rates were also significantly better in patients who had undergone a bilateral subtotal thyroidectomy compared to a Hartley Dunhill procedure (complete excision of one thyroid lobe and isthmus and partial or sub-total excision of the contralateral lobe) (OR 0.35; 95% CI 0.15-0.79; p=0.01) The definitions of hypocalcaemia also varied significantly so meaningful recommendations could not be made (Antakia, Edafe et al. 2014).

As described previously, the parathyroid glands can be difficult to locate accurately at surgery, even for experienced surgeons. There is currently no accepted method of identifying normal parathyroid glands except through visual inspection. There must be a careful balance between looking for the glands to aid preservation and disruption to the vascular supply or direct damage to the glands in the process. Exploration to identify the parathyroid glands can also increase the risk of damage to the recurrent laryngeal nerve. It has also been highlighted that the decision to auto-transplant a parathyroid gland when damaged or devascularised is also at the surgeon’s discretion and the surgeon’s experience and training will influence these decisions. Routine parathyroid auto-transplantation is not recommended (Lo 2002), but glands that do not look viable at surgery are often auto-transplanted. Assessment of viability is subjective, but if technology could guide the surgeon in this decision, then the number of unnecessary auto-transplantations would be reduced, which could reduce the incidence of early hypocalcaemia due to hypoparathyroidism. It would also ensure that patients who would benefit from auto-transplantation receive it and thereby reduce the incidence of long-term hypocalcaemia (PoSH).
There are novel technologies being developed to aid the surgeon in the intra-operative identification of parathyroid glands and their blood supply, so as to improve on human judgement.

Novel technologies tend to fall into two groups; those that focus on intra-operative localisation of the parathyroid glands i.e., screening for the glands or real-time intra-operative tissue differentiation i.e., confirmation that the tissue is parathyroid gland. Intra-operative localisation techniques may work by injection of a substance which preferentially accumulates in the parathyroid gland that can be detected. This includes gamma probes which require injection of technetium Tc 99m-sestamibi 30-60 minutes prior to surgery. This is the same radioisotope used for pre-operative sestamibi scans which aims to localise diseased glands pre-operatively allowing a targeted excision. The gamma signal can be detected during the operation to localise the abnormal parathyroid and assess for multi gland disease using an intra-operative gamma probe. This technology only addresses the localisation of abnormal glands so would not be of benefit to patients undergoing thyroidectomy. There are patients in whom the technetium TC 99m-sestamibi radioisotope does not accumulate within the parathyroid glands where the gamma probe will not work in their case, this may be due to the ratio of oxyphil and chief cells in the parathyroid gland as it appears that oxyphil cell predominance within an adenoma augments Tc 99m uptake (Bleier, LiVolsi et al. 2006). This can be seen in patients who have inconclusive pre-operative imaging who must go on to have a bilateral exploration as their diseased gland could not be localised (Sullivan, Scharf et al. 2001). Freehand SPECT (single photon emission computerised tomography) is another intra-operative imaging system to localise parathyroid adenomas. It works in a similar fashion to the gamma probe, but 3D images can be reconstructed from cross-sectional imaging. It can be difficult to use the images produced to identify the area of interest as they need to be interpreted from a screen and translated to the surgical field but in the future, with 3D overlay technology, this may improve (Rahbar, Colombo-Benkmann et al. 2012). Both of these technologies have their uses, but perhaps with localising ectopic abnormal tissue, as any tissue that is easily localised intra-operatively will also have been able to be pre-operatively localised on imaging. These techniques only aid localisation of abnormal parathyroid tissue. An area of interest that is developing
rapidly is the use of fluorophores in the identification of parathyroid glands. These dyes are injected and accumulate in the parathyroid glands. This technology is discussed in depth later in this chapter.

Intra-operative tissue analysis is often used to confirm tissue type during the operation. In other types of surgery specimens can be sent for a type of histological analysis called fresh frozen section which gives a tissue diagnosis within approximately 20 minutes of the specimen being received in the histopathology department (Novis and Zarbo 1997). This may guide the surgeon to the extent of disease and therefore change the course of surgery e.g., excision margins or detection of metastatic spread (Preeti, Sameer et al. 2016, Nowikiewicz, Śrutek et al. 2019). Fresh frozen section is occasionally used in parathyroid surgery to provide intra-operative confirmation of parathyroid tissue (Dewan, Kapadia et al. 2005). This is expensive and time consuming. The development of intra-operative PTH (ioPTH) measurements to confirm an adequate drop in PTH following excision of the gland had significantly reduced the use of fresh frozen sections (Tampi, Chavan et al. 2014, Aksoy, Adiyaman et al. 2021). These two techniques (frozen section and ioPTH) are not suitable for the assessment of normal glands.

Technologies that are able to give real-time histological information from tissues in vivo without the need for resection would help parathyroid identification and preservation. It may even be possible to differentiate normal and abnormal tissue of the same type. Confocal microscopy and Raman spectroscopy are two such technologies that have been trialled in parathyroid surgery. Confocal microscopy negates the need for tissues to be removed, fixed and stained to be examined microscopically. It can therefore be used to identify parathyroid adenoma from normal tissue and ensure the correct gland is removed (White, Tearney et al. 2000). Raman spectroscopy is based on the way light interacts with molecular vibrations. Therefore, tissues will have different spectroscopic features that will allow them to be differentiated (Palermo, Fosca et al. 2018). These technologies confirm tissue diagnosis but do not aid localisation or preservation of the tissue or vascular supply. Both of these technologies may require expertise for accurate image analysis and have not yet been demonstrated to be effective in clinical practice.
Another technology which may be able to differentiate tissues is electrical impedance spectroscopy. It works by analysing how current moves through tissues and is based on the cellular arrangement of the tissues. If proven to work, the technology would give real time feedback to the surgeon and may not require additional expertise. This is discussed in further detail later in this chapter.

1.6.2 Near infrared fluorescence

Near infrared fluorescence imaging is one of several novel technologies that may be useful in early identification and preservation of parathyroid glands during central compartment neck surgery. Near infrared fluorescence (NIRF) imaging exploits the property of either endogenous or exogenous fluorophores. Fluorophores are molecules that, when excited by a light source, re-emit light of a higher wavelength than the initial stimulating wavelength. Some emit light outside of the visible spectrum in the near infra-red region (700-900nm) (Schafsma, Mieog et al. 2011). Light in the visible spectrum has limited tissue penetration (100-300µm) thus limiting utility for imaging internal organs. Near infrared (NIR) light however penetrates several millimetres through tissues (van der Vorst, Schafsma et al. 2014, Tummers, Boonstra et al. 2015).

NIRF has been used during surgery to aid real-time intra-operative visualisation of tissues and differentiate between tissue types (Matsui, Tanaka et al. 2010, Matsui, Tanaka et al. 2010, Crane, Themelis et al. 2011, van der Vorst, Schafsma et al. 2013, Tummers, Boonstra et al. 2015). Applications include sentinel lymph node mapping, defining cancer resection margins and assessment of biliary anastomoses (Crane, Themelis et al. 2011, Hutteman, van der Vorst et al. 2012, van der Vorst, Schafsma et al. 2013, van der Vorst, Schafsma et al. 2013). Fluorophores that are approved for use in humans include methylene blue (MB), indocyanine green (ICG) and 5-aminolevulinic acid (5-ALA). Intra-operative use of NIRF exploits the routes of metabolism and clearance of these agents in the body. ICG is excreted by the liver and is therefore present in bile. NIRF using ICG has been used to image bile ducts and liver cancers (Gotoh, Yamada et al. 2009, Ishizawa, Fukushima et al. 2009, Matsui, Tanaka et al. 2010, Hutteman, van der Vorst et al. 2011, Lim, Vibert et al. 2014, Sakoda,
Methylene Blue is a good candidate for use in thyroid and/or parathyroid surgery as it is taken up readily by endocrine tissues, though the mechanism for this remains unknown (Hurvitz, Perzik et al. 1968, Dudley 1971). MB has traditionally been used intravenously in high doses (3-7.5 mg/kg) to aid naked eye identification of enlarged parathyroid glands by discoloring the gland blue and is reported to be very sensitive (Patel, Chadwick et al. 2012). However, high dose MB has many disadvantages (Patel, Chadwick et al. 2012). The operative field may be discoloured by the blue dye; the staining may not be visualised if other tissues overlie the stained organ; there may be significant discoloration of the patient’s skin and urine and severe allergic reactions, but this latter side effect is rare. High dose MB must be given as an infusion and there is risk of extravasation of the dye. This has potential catastrophic effects of tissue necrosis due to intense vasoconstriction and free radical cytotoxic cellular injury. At these high doses, MB can also exert neurotoxic effects. MB acts as a monoamine oxidase inhibitor which can lead to serotonin toxicity due to increased intra-synaptic levels of serotonin, especially when used in conjunction with serotonin re-uptake inhibitors (SSRIs) adding significant morbidity to an otherwise relatively safe procedure (Vutskits, Briner et al. 2008, Patel, Chadwick et al. 2012). SSRIs are the most widely prescribed antidepressant medication, it is the first line antidepressant of choice recommended in the National Institute for Clinical and Health Excellence (NICE) Guidance for treatment of depression in adults (CG90), and it is possible that a proportion of patients coming for surgery will be prescribed this medication. MB is rapidly absorbed in nervous tissue and rodent models have shown brain concentrations to be 10 times higher than serum levels (Top, Gillman et al. 2014). G6PD deficiency is an X-linked recessive condition and is the most common enzymatic deficiency in red blood cells. MB must not be used in patients with this condition as MB may induce haemolysis. High dose MB has also only been used to
identify enlarged, abnormal glands and has not been shown to be effective in the identification and preservation of normal glands.

MB emits light in the near infrared range (~700 nm (Gioux, Choi et al. 2010)); this property can be exploited to use it at much smaller doses. As NIR light is invisible to the naked eye, the dye does not stain the operating field. Its presence is detected by a camera with a filter to block light of other wavelengths and is projected to a screen for visualisation (Figure 1.5). An additional advantage is that NIR radiation penetrates tissue better than visible light.

ICG has a peak absorption wavelength of 805 nm and re-emission wavelength of 835 nm. It binds irreversibly to plasma proteins in the blood and circulates in the intravascular compartment only. It is a useful agent for angiography and has been utilised in studies assessing parathyroid viability following thyroidectomy (Vidal Fortuny, Belfontali et al. 2016, Zaidi, Bucak et al. 2016). 5-ALA is an endogenous compound that is converted into a fluorescent substance called protoporphyrin IX (PpIX), it can be administered exogenously to enhance the substrate concentration. 5-ALA has been found to accumulate in parathyroid tissue. It can be administered orally, and fluorescence is seen at 1 hour after administration, peaking at 4-6 hours depending on dose. Blue light is used to illuminate the tissues and PpIX fluoresces red (Takeuchi, Shimizu et al. 2014). It has potential to aid parathyroid localisation but the long lag from administration to peak fluorescence means timing of administration is difficult to ensure peak fluorescence is achieved while the patient is on the operating table.

Parathyroid glands have also been shown to auto-fluoresce within the near-infrared spectrum. Although the mechanism of auto-fluorescence is not understood, a study of 21 patients reported in 2011 showed that when using an excitation wavelength of 785 nm the parathyroid glands fluoresced more brightly than the thyroid or other neck structures (Paras, Keller et al. 2011). The fluorescence appeared to be independent of parathyroid pathology and the glands had a fluorescence intensity two to eleven times greater than that of the thyroid. However, the fluorophore that causes auto-fluorescence in parathyroid glands does not appear to be dependent on
gland viability as ex vivo glands continue to exhibit the same properties (McWade, Paras et al. 2013).

In the study described in this thesis (Chapter 2) Methylene blue was selected as the fluorophore of choice as due to its uptake into parathyroid tissue, as opposed to ICG which remains intravascular. The technology developed by Fluoptics is compatible with the use of Methylene blue due to the wavelengths required for fluorophore excitation. The equipment used in this study was on loan from Fluoptics and they were available for technical support but did not participate in the study design or execution.

**Figure 1.5  Principles of Methylene Blue Near Infrared Fluorescence**

*The MB molecule is excited by the laser at a wavelength of 680nm. MB emits light at around 700nm. This is captured by the camera which has a filter to block out any light below 700nm so only light emitted from the MB molecule is collected.*
1.6.3 Electrical impedance spectroscopy

The histological appearances of the thyroid and parathyroid glands are different, enabling easy differentiation between the two when examined under the microscope. The thyroid gland is comprised of follicles which consist of a single layer of secretory cells with a central cavity where the hormone is stored. The inactive form of thyroid hormone is bound to glycoprotein in these cavities making colloid. Active glands have a different appearance to inactive glands. The secretory cells have a columnar appearance when active as they are packed with endoplasmic reticulum and golgi bodies. The colloid cavities in this state are smaller and therefore the overall size of the follicles are smaller. In the inactive form, secretory cells have a low cuboidal appearance with large colloid stores creating large follicles. The thyroid has a rich blood supply and parafollicular or clear cells that produce calcitonin.

The parathyroid glands in comparison are densely packed glands and consist of two cells: chief and oxyphil cells. Chief cells manufacture and secrete parathyroid hormone (PTH). When active and full of hormone they have a darker cytoplasm than in their resting state when they are pale. The two tissues can be compared in Figure 1.6.

Figure 1.6  Hematoxylin and eosin stained histological specimen of thyroid and parathyroid tissue

A) The thyroid shows the follicles containing colloid surrounded by a thin layer of cells. B) The cells of the parathyroid tissue are densely packed and lack follicles.
Electrical impedance (EI) is the measure of resistance to an alternating current (AC) in a circuit. Resistance is a term used when using direct currents (DC) and is a measure of the opposition to current flow in an electrical circuit. Electrical impedance is used when an alternating current is applied as both resistive and reactance effects the opposition of an alternating current. If a material, or biological tissue is placed as part of the circuit then the resistance of the intervening tissue can be measured. Electrical conduction in biological tissues is due to ions, unlike in metallic conductors, and conduction develops due to the movement of mobile ions within the aqueous biological medium (Dean, Ramanathan et al. 2008). Biological tissues have both resistive and capacitive properties and therefore have complex impedance. This is determined by the presence of ions, which may be bound in cell membranes whilst others are free to move. The capacitive components of a tissue will store charge when a current is applied. Then the potential is removed the stored charge will be dispersed through the resistive components (Brown, Highfield et al. 2020). Factors that affect the resistance include tissue architecture, intracellular content and cell adhesion or extracellular space. At low frequencies (<100 Hz), the current must pass through the extracellular space and therefore cell arrangement is the greatest factor in resistance. At higher frequencies (>1 GHz), the current is able to penetrate the cell membrane and therefore the cellular contents become important in influencing resistance (Figure 1.7). EI is also dependent on ion mobility, which is affected by temperature-induced fluid and electrolyte shifts within the tissues. By applying a range of frequencies and measuring the impedance of the tissue at each frequency, an impedance spectrum is created that will be unique for that tissue (Brown, Tidy et al. 2000).
In addition to the architectural differences between different tissues, morphological changes also occur during disease processes such as neoplasia. The internal cell structure tends to alter with a different nuclear-cytoplasmic ratio. The arrangement of the cells also becomes less structured and the extracellular space increases (Brown, Tidy et al. 2000). These changes lower the resistance of the tissue, and this finding has clinical applications in the identification of cancerous or pre-cancerous lesions. This has been tested in cervical, breast, prostate, bladder and skin cancers (Stojadinovic, Nissan et al. 2008, Aberg, Birgersson et al. 2011, Keshtkar, Salehnia et al. 2012, Mishra, Bouayad et al. 2012, Mishra, Schned et al. 2013).

Electrical impedance spectroscopy (EIS) is used as an adjunct to traditional colposcopy (Brown, Highfield et al. 2020). Brown et al used an EIS probe to systematically test the impedance of the cervical epithelium across eight frequencies in eight locations on the cervix prior to the standard cervical biopsy (Brown, Milnes et al. 2005). This study showed that sensitivity and specificity of 80-90% can be

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Figure 1.7  Principles of electrical impedance of a tissue

Normal cells within a tissue (a) are tightly packed and well organised so current passing through the extra cellular space is held up in comparison to the neoplastic tissue in (b) which is disorganised with looser cellular connections. The impedance is therefore greater in (a) than (b). At higher frequencies the current penetrates the cell membranes and therefore the difference in nucleus to cytoplasmic ratio in normal and abnormal tissue affects impedance.
achieved in detecting changes associated with the precancerous lesions of cervical intra-epithelial neoplasia (CIN) grade 2 or 3. CIN is graded from 1 to 3 on the depth of cell change. CIN 2 or 3 is when two-thirds or the full thickness of the epithelial layer is affected (Brown, Milnes et al. 2005). Overall, EIS showed similar sensitivity and specificity to existing screening methods. The advantage of EIS over current methods is the potential for instant results that could allow diagnosis and treatment in one appointment. A subsequent multicentre trial by Tidy et al showed that EIS as an adjunct to colposcopy can improve the accuracy of detection of high-grade disease. The positive predictive value increased from 78.1% (95% CI 67.5–86.4) to 91.5%. Specificity was also increased from 83.5% (95% CI 75.2–89.9) to 95.4%, but sensitivity was significantly reduced from 73.6% (95% CI 63.0–82.5) to 62.1%, and the negative predictive value was unchanged (Tidy, Brown et al. 2013).

EIS has been evaluated as a non-invasive method of identifying individuals who are not already identified as high risk for breast cancer but may benefit from earlier screening. The device scans over nine segments of a clinically normal breast and detects any changes in the breast parenchyma associated with malignancy (Stojadinovic, Nissan et al. 2008). This technology does not produce an image or give any further information on the abnormality and therefore does not allow diagnosis. It is used as a prompt that the individual may require further imaging. A patient with a positive scan was five times more likely to have breast cancer at the time of examination compared to a patient with a negative scan (Stojadinovic, Nissan et al. 2008).

EIS has been studied in ex vivo human prostates (Mishra, Bouayad et al. 2012, Mishra, Schned et al. 2013). A needle EIS probe is inserted into the prostate. Prostate cancer is highly glandular, and the impedance of this tissue is shown to be higher than that of normal or benign prostate tissue. Therefore a change in the spectra has potential to identify areas of concern and direct the clinician to the area to be biopsied (Mishra, Schned et al. 2013).

EIS has been studied in the identification of malignant melanoma (Aberg, Nicander et al. 2004, Malvehy, Hauschild et al. 2014) and compared to traditional diagnosis with a
dermoscope, a high-powered magnifier. Studies concluded that EIS could distinguish between benign and malignant lesions but that the sensitivity to thin malignant melanomas needs to be improved (Aberg, Birgersson et al. 2011). In comparison to the use of dermoscopes, EIS was no better in the hands of experts but showed significant improvement on diagnosis in novices (Aberg, Birgersson et al. 2011).

EIS has been tested in thyroid diseases to ascertain the nature of indeterminate thyroid nodules. Nissan et al used an EIS probe on the skin overlying thyroid nodules of ≥5 mm in patients scheduled for thyroid surgery (Nissan, Peoples et al. 2008). Current diagnostic modalities for thyroid nodules are ultrasound, fine needle aspiration, radiiodine scanning and diagnostic thyroid lobectomy. This study aimed to investigate EIS as a non-invasive method of differentiating benign from malignant nodules with a view to reducing the number of diagnostic operations for benign conditions. 216 patients underwent fine needle aspiration (FNA) and EIS pre-thyroidectomy. EIS results were either classed as malignant or non-malignant and were correlated with final histopathology results. EIS correctly diagnosed 96 of 110 malignant nodules and 75 of 106 patients with benign dominant thyroid nodules. The investigators found that EIS could safely be used as an adjunct to current diagnostic evaluation, and state that EIS would significantly reduce the number of operations performed for benign nodules (71%) and also significantly reduce the number of purely diagnostic thyroidectomy for indeterminate FNA results (67%). (Nissan, Peoples et al. 2008).

These studies are based on the principle that impedance is reduced in malignant tissues compared to benign tissues within one tissue type. The same principle could be applied to different tissue types if the impedance of the tissues is significantly different. As the structure of the thyroid and parathyroid glands are significantly different, it has been hypothesised that the impedance spectra of the tissues would also be significantly different. This may have the potential to be used as an intra-operative aid to differentiate between a parathyroid gland and other tissues such as lymph node or thyroid nodule.
1.7 Hypothesis, aims and objectives

Novel techniques are able to assist in intraoperative localisation of the parathyroid gland and may reduce rates of PoSH and enhance quality of life after neck surgery.

1.7.1 Phase 1b clinical trial – near infrared fluorescence

The aim of this study was to develop a protocol for the use of Fluobeam® 700 in combination with intravenous methylene blue, for the detection of parathyroid tissue with differentiation from adjacent soft tissue, during thyroid and parathyroid gland surgery.

The objectives were to:

- identify the optimum dose of intravenous methylene blue and time to peak fluorescence of normal and abnormal thyroid and parathyroid glands
- to determine differences in patterns (onset, intensity, and duration) of fluorescent staining between the various soft tissues of interest in the neck.

1.7.2 Phase 1b clinical trial - electrical impedance spectroscopy

The aim of this study was to evaluate the potential for electrical impedance spectroscopy to be used in human thyroid and parathyroid surgery.

Specific objectives were:

- To determine EI spectra of normal and abnormal human thyroid, parathyroid, lymph nodes, fatty tissue and skeletal muscle.
- To compare the EI patterns of the parathyroid with the other soft tissue structures in the human neck.
- To investigate changes in EI before and immediately after excision of soft tissue structures in thyroid and parathyroid surgery.
- To develop a protocol for the use of EI in the accurate differentiation of parathyroid glands from other structures including thyroid nodules, adipose tissue and lymph nodes.
1.7.3 Assessment of quality of life in patients with PoSH

The aim of the study was to perform a detailed assessment of symptoms and quality of life in different cohorts of patients with post-surgical hypoparathyroidism and to compare these patients with similar cohorts of patients without hypoparathyroidism. This study takes into account the aetiology of hypoparathyroidism, treatment and co-morbidities to analyse for possible predictive factors.

1.7.4 Post-operative calcium levels and its management following parathyroid surgery

To evaluate the risk of transient and long-term post-operative hypocalcaemia and/or hypoparathyroidism rates following parathyroid surgery and to validate (or amend) the existing guidelines on the management of transient post-surgical hypoparathyroidism.

Specific objectives for this study are:

- To evaluate transient and long-term hypocalcaemia/hypoparathyroidism rates against nationally reported figures.
- To assess adherence to existing local guidelines and identify variation and potential reasons for variation from the guidelines.
- To identify any areas where improvements could be made to reduce the rate of post-operative hypocalcaemia (by evaluating risk factors and surgical strategies).
Chapter 2: Near Infrared Fluorescence Imaging

in Thyroid and Parathyroid Surgery
2.1 Background to the study

Identification of parathyroid glands is important in both thyroid and parathyroid surgery. Preservation of normal glands is essential to maintain calcium homeostasis and excision of abnormal glands is key to the treatment of primary hyperparathyroidism. In thyroid surgery, a proportion of surgeons aim to identify parathyroid glands in order to preserve the gland and the associated blood supply in addition to preventing post-operative hypocalcaemia. Others do not routinely identify all glands but limit their dissection to the capsular plane to avoid inadvertent injury. Despite these approaches, parathyroid injury and/or devascularisation continues to occur during operations of the central compartment (as discussed previously in section 1.3). The parathyroid glands, as previously described (section 1.1), can have a varied position and their size and delicate nature increases the risk of bruising and devascularisation. Parathyroid glands may also be mistaken for other soft tissue structures in the central neck compartment such as lymph nodes or thyroid nodules, or vice versa.

Near infrared fluorescence is a novel technology which has the potential to be used as an adjunct in thyroid and parathyroid surgery that could prevent post-surgical hypoparathyroidism. Near infrared fluorescence (NIRF) imaging uses fluorophores which are dyes that re-emit light of a higher wavelength (but lower energy) when excited by a light source. This emission occurs anywhere along the electromagnetic spectrum, with a proportion outside of the visible spectrum and in the near infrared region (700-900nm) (Schaafsma, Mieog et al. 2011). White light or light in the range used for human vision, has poor penetration of tissues and therefore only the surface of tissues is visible to the eye. A tissue emitting white light would only require a thin covering of other tissue to obstruct the light from view. As the wavelength increases, the penetration through tissues also increases and near infrared light can travel several millimetres through tissues (van der Vorst, Schaafsma et al. 2014, Tummers, Boonstra et al. 2015, Ash, Dubec et al. 2017). Therefore, tissue emitting light in the near infrared region of the spectrum can be detected through thin layers of normal tissue. Fluorophores that are currently approved by the Federal Drug Administration (FDA) and the European Medicines Agency (EMA) for clinical use include methylene
blue (MB), indocyanine green (ICG) and 5-aminolevulinic acid (5-ALA). Current uses of NIRF imaging include sentinel lymph node mapping, defining cancer resection margins and assessment of biliary anastomoses (Crane, Themelis et al. 2011, Hutteman, van der Vorst et al. 2012, van der Vorst, Schaafsma et al. 2013, van der Vorst, Schaafsma et al. 2013).

Methylene blue, or methylthioninium chloride, is a thiazine dye which was first synthesised in 1876 (Cwalinski, Polom et al. 2020). It has been used in many ways since its discovery and can be administered topically, orally, subcutaneously or intravenously to enable clinicians to examine its dispersion and thereby delineate anatomy. Surgical uses include the mapping of intestinal or enterocutaneous fistulae and in the identification of sentinel lymph nodes. As previously described (section 1.6.2), methylene blue is known to be taken up into endocrine tissues readily. Traditional use in endocrine surgery has been high dose (3-7.5mg/kg) intravenous administration to aid naked eye identification of enlarged parathyroid glands by discolouring the gland blue. The mechanism by which parathyroid glands take up and retain MB is not understood, but the first report of using methylene blue to visualise parathyroid glands dates back to 1971 (Dudley 1971). The parathyroid glands became discoloured over the period of an hour and remained stained for approximately two and a half hours. It was also shown that normal parathyroid glands took up the methylene blue differently to parathyroid adenomas; the former stained dusky blue, whereas in the latter, the glands were dark blue to purple (Dudley 1971). Methylene blue is efficacious in identifying enlarged glands; a systematic review by Patel et al. showed that 100% of abnormal glands demonstrated staining following administration of intravenous methylene blue (Patel, Chadwick et al. 2012). Methylene blue has a long half-life of 5-6.5 hours and is excreted via the kidneys. However, high dose methylene blue has many disadvantages. The operative field may be discoloured by blue dye; the staining may not be visualised beneath other tissues; there may be significant discolouration of the patient’s skin and urine; and, though rare, patients may suffer severe allergic reactions to the dye. At these doses, methylene blue has also been shown to exert neurotoxic effects, which is exacerbated when used in conjunction with serotonin re-uptake inhibitors (SSRIs), adding significant morbidity to an otherwise relatively safe procedure (Vutskits,
Briner et al. 2008, Patel, Chadwick et al. 2012). Of the fluorophores available currently, methylene blue appears to be favourable for use in thyroid and/or parathyroid surgery due to its uptake into endocrine tissues (Hurvitz, Perzik et al. 1968, Dudley 1971).

Methylene blue can be used as a fluorophore, with light emissions in the near infrared range (~700nm). Much smaller doses of methylene blue are required for this and therefore the risks associated with high dose methylene blue are reduced. Methylene blue will be taken up into the parathyroid tissue as with the high dose intravenous administration, but the tissues will not change colour. Instead, the fluorescence is detected with an imaging system.

Parathyroid auto-fluorescence in the near infrared spectrum has been observed in several studies (Paras, Keller et al. 2011, De Leeuw, Breuskin et al. 2016, McWade, Thomas et al. 2019) using wavelengths of 700nm and 785nm. The parathyroid appeared 2-11 times brighter than the surrounding thyroid and soft tissue. The mechanism behind auto-fluorescence has not yet been identified but the fluorescence appears independent of parathyroid pathology and blood supply, as ex vivo glands continue to exhibit the same properties (McWade, Paras et al. 2013). The Fluobeam® 700 (Fluoptics, Grenoble, France) is a portable NIRF imaging system comprising of a control box linked to a camera head (Figure 2.1). The camera head contains a class 1 laser (wavelength 680nm), white light emitting diodes (LED, wavelength 390-700nm) and a charge-coupled device camera.
The white light LEDs illuminate the surgical field while the laser excites the methylene blue within the tissues, generating fluorescence. The camera has a high-band pass filter, which ensures that only fluorescent light over 700nm is collected (Figure 2.2). The control box is linked to a laptop where real-time still and video images are displayed on screen and recorded. The camera functions including exposure, control of the laser and LEDs and start/stop image capture are controlled from the software on the laptop. The camera has a button which can switch the laser on and off for safety.

The hypothesis that methylene blue near infrared fluorescence could be used in thyroid and parathyroid surgery has been tested in a rabbit model in our laboratory (Antakia, Gayet et al. 2014). Rabbits were chosen as it is easy to identify the glands of interest as they have an “external” parathyroid gland on each side of the neck so both the parathyroid and thyroid glands can be identified on neck dissection. Six New Zealand rabbits under general anaesthesia had their thyroid and parathyroid glands

**Figure 2.1  Fluobeam 700**

*Left:* Fluobeam® 700 arranged for use in theatre. The control box (middle) is linked to a screen or laptop with Fluosoft™ installed. The camera head is stored in the basket below. *Right:* Fluobeam® 700 camera head.
exposed. Varying doses of methylene blue were injected via a vein and the fluorescence of the thyroid and parathyroid glands were studied over time. The patterns of fluorescence, fluorescence intensity, onset and duration were investigated. Serial bolus doses were administered of increasing concentration, once the fluorescence from the glands from the previous dose had returned to near baseline. The glands were confirmed by histological analysis on completion of the experiment. This study showed that both thyroid and parathyroid glands fluoresced at low doses (0.025-3mg/kg) of methylene blue, but a minimum dose of 0.1mg/kg was required to differentiate the glands adequately. Overall, the parathyroid glands showed a lower intensity of fluorescence with faster washout when compared to the thyroid gland. As the dose of methylene blue increased, the difference in peak intensity between the thyroid and parathyroid glands also increased (Figure 2.3) (Antakia, Gayet et al. 2014). This study provided “proof of concept” and aided the design of human studies including estimated dose requirements. One potential

Figure 2.2  Principles of fluorescence imaging

A class 1 laser in the camera head is the light source which excites the methylene blue molecule. This emits light in the near infrared region and is detected by the camera. An emission filter excludes light of other wavelengths from detection.
problem the study highlighted was that the glands were easy to differentiate with fluorescence in real time but when it came to post-procedure analysis it was notably harder, especially when the glands were in close proximity.

A Phase IA human study was designed to evaluate the feasibility of using NIRF imaging in the detection of parathyroid tissue, to familiarise the surgical team with the intra-operative use of the Fluobeam® 700 device and to evaluate the patterns of fluorescent staining of soft tissues of the neck during parathyroidectomy by our group (ClinicalTrials.gov 2012). This study showed that methylene blue fluorescence was detected during neck surgery, with differences in fluorescence between thyroid, parathyroid and other soft tissue structures in the neck. Use of the Fluobeam® 700 during surgery was ergonomically feasible and did not hamper the course of the operation. Although methylene blue fluorescence from human thyroid and parathyroid tissue has been reported by other research groups (van der Vorst, Schaafsma et al. 2014, Tummers, Schepers et al. 2015), systematic evaluation of dose response and temporal changes in fluorescence has not been performed.

![Figure 2.3](image)

**Figure 2.3**  Methylene blue fluorescence from thyroid and parathyroid glands in rabbits

*Methylene blue near infrared fluorescence in rabbit thyroid and parathyroid glands over time with increasing concentrations of methylene blue.*
2.2 Aims

The aim of this study was to develop a protocol for the use of Fluobeam® 700 in combination with intravenous methylene blue, for the detection of parathyroid tissue with differentiation from adjacent soft tissue, during thyroid and parathyroid gland surgery.

The objectives were to identify the optimum dose of intravenous methylene blue and time to peak fluorescence of normal and abnormal thyroid and parathyroid glands and to determine differences in patterns (onset, intensity and duration) of fluorescent staining between the various soft tissues of interest in the neck.

2.3 Methods

2.3.1 Study design

This is a Phase 1b interventional study, without a control arm. Two groups of patients were included - patients with primary hyperparathyroidism (PHPT) undergoing parathyroidectomy (bilateral neck exploration, unilateral neck exploration or targeted parathyroidectomy) and patients undergoing thyroid resection (hemithyroidectomy or total thyroidectomy). Whilst patients undergoing parathyroidectomy had surgery for primary hyperparathyroidism, thyroidectomy was performed for a number of reasons: multinodular goitre, thyroid cancer, benign cysts or Graves’ disease for example. By including both patients undergoing thyroid and parathyroid surgery, both normal and abnormal glands were included in the study. This was deemed a Phase 1b study following discussion at the ethics committee meeting as it is a study to determine the appropriate dose for use in humans before proceeding to a Phase 2 study and to differentiate it from the Phase 1a study in rabbits performed previously.

2.3.2 Ethical approval

The study was approved by the Medicines and Healthcare Regulatory Agency (MHRA) (Reference number: 21304/0252/001-0001) and the Regional Research Ethics Committee (REC) (Reference number: 14/NW/0270). The study protocol was
registered with ClinicalTrials.gov (ClinicalTrials.gov 2014). All patients included in the study were fully informed and gave written consent.

This study considered Methylene blue as a clinical trial of an investigational medicinal product (CTIMP) which required a higher level of scrutiny by both MHRA and REC. This included clinical trial insurance and working with the clinical trials department in Pharmacy as the methylene blue needed to be dispensed on a named basis and quarantined as per CTIMP protocol. The MHRA made recommendations to the exclusion criteria outlined below to maintain patient safety with the use of MB.

The Fluobeam® 700 device was on loan from Fluoptics for the study and were available for technical assistance. They did not participate in the study design or analysis and there are no conflicts of interest to declare.

2.3.3 Participant eligibility

All patients aged 18 and older undergoing either a total thyroidectomy or hemithyroidectomy for thyroid disease or bilateral neck exploration, unilateral neck exploration or targeted parathyroidectomy for PHPT in Sheffield Teaching Hospitals NHS Foundation Trust were eligible for the study.

There were a number of exclusion criteria:

- Patients unable to understand spoken and written English,
- Patients unable to give adequate informed consent,
- Patients with a history of intolerance or sensitivity to methylene blue,
- Patients with known G6PD deficiency,
- Patients undergoing re-do procedures,
- Patients undergoing surgery for thyroglossal cyst,
- Patients undergoing thoracic exploration; either alone or in combination with a neck exploration.
- Patients on serotonin reuptake inhibitors or taking any medicinal products that enhanced serotonergic transmission including for example SSRIs, bupropion, buspirone, clomipramine, mirtazapine and venlafaxine.
- Patients who were pregnant or breastfeeding.
- Patients with an eGFR of $\leq 59 \text{ ml/kg/1.73m}^2$ (eGFR was determined as part of routine work up before surgery and the last available measurement was used to make this decision)

In addition, the surgeons excluded certain patients from the study at their discretion if the case was considered complex and it was felt that the study intervention would hinder the progress of the operation.

### 2.3.4 Approved updates to the study protocol

The original inclusion criteria only included those undergoing a total thyroidectomy or bilateral neck exploration. The academic steering group reviewed this after the first three months of recruitment after two patients had participated in the study. Recruitment was slow and the number of patients declining consent to the study was high. On review of the data from the first two patients it was apparent that only one side of the neck could be visualised in detail by the camera following MB injection. This allowed the inclusion criteria to be expanded to include patients undergoing a unilateral procedure and therefore increased the pool of patients without affecting the data collected. The eligibility criteria were updated and approved by REC in April 2015 to include patients undergoing total thyroidectomy or hemi-thyroidectomy and patients undergoing bilateral neck exploration, unilateral neck exploration or targeted parathyroidectomy for PHPT.

The second issue raised by patients declining to participate in the study, was that the potential additional time of 30 minutes under general anaesthesia was too long. The data from the first two patients showed that the planned 20-minute recording time after the first bolus was not necessary as the peak of fluorescence was seen in the first minute and fluorescence had returned to baseline within 10 minutes. The original protocol stipulated that a second bolus could be given which would extend the anaesthetic time further and the research team felt that this second bolus would not be of benefit in view of any potential cumulative effect of two boluses within a short time period. Therefore, a second amendment was made to the protocol to reduce the number of boluses given to one and the recording time was limited to 10 minutes. With these changes, recruitment to the study was notably improved.
2.3.5 Participant recruitment

Patients undergoing thyroidectomy or parathyroidectomy between November 2014 and May 2016 at Sheffield Teaching Hospitals NHS Foundation Trust (STHNFT) were identified either in the endocrine surgical outpatient clinic at the time of their placement on the waiting list or in pre-assessment clinic prior to their operation. At the initial consultation, the patients were given an explanation of the study and an information leaflet with further details. Patients were then approached later by a member of the research team for a second consultation when the study was discussed in further detail and the patient was given an opportunity to ask further questions. This second consultation was held either in pre-assessment clinic or the theatre admissions unit on the morning of their operation. If the patient was willing to participate, written consent was then taken.

2.3.6 Study phases

The study was divided into three phases to facilitate the decision-making process for dosing and to address the different aims of the study. These stages were named ‘training’, ‘testing’ and ‘final’ phases. The aim of the training stage was to estimate the optimal dose of methylene blue, the duration of observation and to develop a working protocol. The dose of methylene blue was refined in the testing stage before moving onto the final stage where the agreed protocol was tested. In the training and testing stages, methylene blue doses starting at 0.05mg/kg were administered and increased in increments of 0.05 mg/kg up to a maximum of 0.5 mg/kg in subsequent patients. There are no formal statistical grounds (e.g., precision or power) on which an informed decision could be made regarding a sample size. A sample size of 50 (25 of each procedure) was estimated based on pragmatic grounds, keeping in mind the design of the protocol. Thyroid and parathyroid patients have been included to ensure a range of both normal and abnormal glands as the uptake and retention of methylene blue may differ across pathologies.
2.3.7 Researcher Training

A training day was arranged at the Royal Hallamshire Hospital, Sheffield. This was led by Stephanie Guillermet, Product and Clinical Application Manager at Fluoptics, and was attended by the researchers, anaesthetists, lead theatre practitioners, staff from Research and Development and the Clinical Research Facility. The aim of the training day was to familiarise the research team with the equipment and to produce an intra-operative checklist. The team were taught to assemble the equipment and to run safety checks to ensure the camera was working correctly. The product includes a class 1 laser, a low powered device which is safe for the naked eye. These lasers are inherently safe with no possibility of eye damage therefore, no further signage or protective equipment was required. The group was taught how to apply the sterile cover for intra-operative use. Staff were then introduced to the Fluosoft™ software including variables that can be changed intra-operatively such as exposure and image capture. It was decided at this meeting that an exposure setting of 83ms should be used as this is the standard setting recommended by Fluoptics and an image capture setting of 200ms. The exposure setting could be adjusted intra-operatively to improve the definition of images captured. Fluobeam® 700 requires low level ambient light to gain the best fluorescent images, similar to that when performing a laparoscopic procedure. To try and standardise the theatre light levels, it was agreed that only one operating light would be switched on and upturned during recording. This was deemed a safe level of lighting for theatre staff to work but also to capture the best quality images for the study. The theatre team had an opportunity to practise assembling the equipment and using the software. A user guide was provided, and a quick start guide was written by the clinical research nurse to be used intra-operatively.

All members of the research team who would use the camera, software, make investigational medicinal product (IMP) dose calculations or administer the IMP had Good Clinical Practice (GCP) certificates at the time of the study.
2.3.8 Investigational Medicinal Product (IMP) preparation

0.5% w/v Methylene Blue (Provepharm’s Methylene Blue Injection EP, “Methylthioninium chloride Proveblue” 5 mg/mL, Solution for injection) was used. This is supplied as 10 ml ampoules that contain 50mg Methylthioninium chloride per ampoule. Supplies were kept in pharmacy who were alerted that a potential participant was expected the following day. Once the patient had been consented to participate a completed prescription form was taken to pharmacy and the IMP was dispensed and labelled for clinical trial. Methylene blue is administered undiluted as a slow bolus. The cannula is flushed with 5% glucose IV solution. Unused Methylthioninium chloride was discarded, and the product packaging was returned to pharmacy. Unopened vials of methylene blue were returned to pharmacy and quarantined.

2.3.9 Intra-operative methods

At thyroid and/or parathyroid surgery under general anaesthesia, the intervention was paused after exposure of the thyroid gland and at least one parathyroid gland to assess fluorescence. Accurate identification of the parathyroid gland relied on the surgeon’s judgment based on the appearance and location of the gland. In patients undergoing parathyroid surgery, the abnormal gland was identified. If the patient was undergoing a bilateral procedure, only one side of the neck was included in the recordings and assessments. The study could only proceed after at least one parathyroid gland was identified with confidence by the surgeon (Figure 2.4).

The Fluobeam®700 screen was positioned on the opposite side to the person holding the camera for ease of viewing (Figure 2.5) At this stage, the Fluobeam® 700 camera in a sterile, transparent cover and connected to a laptop computer was held at a working distance of 20 cm from the surgical field. The sterile cover was redesigned part way through the study. The original end of the cover had been designed to include a special clear disc that was aligned over the camera, but this produced a periscope effect on the screen, reducing the amount of the screen that was in focus.
**Figure 2.4** Intra-operative view of thyroid and parathyroid glands

Abnormal thyroid retracted medially by the surgeon. Normal parathyroid identified

**Figure 2.5** Intra-operative use of near infrared fluorescence

The screen is positioned opposite the surgeon using the Fluobeam 700® camera on the same side as the gland identified at a working distance of 20cm.
This was rectified and a new cover that was clear over the entire end of the camera was introduced, and the quality of the images improved significantly. The ambient room light was dimmed to a level similar to that used during laparoscopic surgery and the camera set at an exposure time of 83ms. For the first patient in the study, an interval of 200ms between images was initially chosen. This produced too many images over the course of the recording to allow analysis in a timely fashion to be able to make decisions on the dose for the subsequent patient and little information was gathered from the additional images. The interval between images was then increased to 1000ms. At this setting, processing of images and analysis of fluorescent patterns to determine doses in subsequent patients took around 6 hours.

Recording was started before methylene blue was infused and was stopped at 10 minutes after injection except in one patient (Study Identification (SID) 028) where recording was stopped early as no fluorescence was seen due to parathyroid ischaemia. The parathyroid gland was highlighted for identification purposes post procedure at the start of the recording (usually with forceps) and then other surgical instruments were removed from the surgical field to improve clarity of the images. The administration of the bolus was initially indicated for the recording by the passing of a hand in front of the camera. However, this was considered unreliable as it may be missed on the image capture if the hand moved too quickly. Therefore, the bolus was given at a set time point of around 10 seconds after the initiation of recording. This allowed enough time to identify the glands for the recording and then administer the bolus once the surgical field was cleared of instruments. Intravenous methylene blue was then administered at a pre-determined dose based on results from previous participants in the study.

The dose of methylene blue to be given was based on the patient’s weight recorded in the pre-assessment clinic and was independently checked by a second member of the research team. For the first patient, a dose of 0.05 mg/kg body weight of methylene blue was administered based on extrapolation from the previous animal studies (Antakia, Gayet et al. 2014). The changes in dose for subsequent patients depended on the results obtained from previous patients in the trial and the nature of the surgery (thyroid vs parathyroid) and were decided upon by the primary
investigators (SLH and SPB). The cannula was primed with 5% dextrose and then methylene blue was administered as a slow IV bolus followed by a flush of 5% dextrose.

Continuous ECG and saturation monitoring was used in all patients. Blood pressure was monitored at 5-minute intervals throughout the operation.

Image J (Schneider, Rasband et al. 2012) was used post-acquisition as a method of quantifying the fluorescence detected from the images obtained (Rasband 1997-2016). A mean greyscale measurement of the pixels within a chosen area is a representation of fluorescence. Fluorescence intensity readings were taken from the thyroid gland, parathyroid gland, soft tissue (muscle or subcutaneous tissue) and surgical drapes. This was a labour-intensive process that could not be easily automated. A free hand selection tool was used to outline the parathyroid gland in each of the approximately 600 images and then this process was repeated for additional parathyroid glands, thyroid, soft tissues and drapes. The images were occasionally degraded by surgical instruments or hands entering the field of view and obscuring all or part of a gland. In this case, an area was selected which best represented the gland. The mean grey scale measurement from the defined area was plotted against time. Ratios were calculated of the thyroid and parathyroid glands compared to soft tissue and the drapes and also plotted against time.

Further analyses were limited to the first 120 seconds of recording in patients given the final dose of 0.4 mg/kg methylene blue. This captured the onset and first peak of fluorescence in all cases. As recording continued past five minutes there is a significant increase in artefact from repositioning of hands and instruments and camera movement. Further peaks after this time were disregarded. Onset and peak fluorescence readings were taken from the first 120 seconds after administration of methylene blue. The first 10 seconds of recording, prior to administration of methylene blue, was used to evaluate parathyroid auto-fluorescence.

Data analyses were primarily descriptive. The time to peak fluorescence of the thyroid and parathyroid glands were compared using the Wilcoxon signed rank test and the fluorescent ratios were compared using the Wilcoxon signed rank test.
Patients undergoing thyroid and parathyroid surgery would routinely have the excised tissue sent for histological assessment and have a post-operative serum calcium level the morning after their surgery as an inpatient. These results were reviewed along with the patient’s discharge summary and the clinical letter from their follow up outpatient appointment for diagnosis and management of hypocalcaemia.

2.4 Results

2.4.1 Demographics

A total of 101 patients who were due to undergo thyroid and/or parathyroid surgery were screened for eligibility, approached for participation and given study information leaflets. A second meeting was planned to confirm eligibility and consent. 41 patients participated, 2 patients were not interested in participating at the first meeting and were not met again, 7 patients declined consent, 22 patients were not eligible to participate, 28 patients were unable to participate due to scheduling (either unable to arrange a second meeting or the research team were unavailable on the operation date), and 1 patient was withdrawn from the study (Figure 2.6). An eGFR ≤59 ml/kg/1.73m² (n=4) or use of an SSRI (n=7) were the main reasons patients were not eligible to participate in the trial. Other reasons included pregnancy, inability to give informed consent, abnormal pre-operative vocal cord check and complexity of the operation. The latter two reasons were at the discretion of the operating surgeon.

Of the 42 patients (36 female, 6 male), 32 underwent surgery for thyroid pathology and 10 for primary hyperparathyroidism. The median age of participants was 49 (range 20 – 85). One patient (SID 024) was withdrawn prior to administration of methylene blue as a decision was made not to interrupt the surgery due to respiratory difficulties that were encountered intra-operatively and was therefore not included in the study. Two patients’ results were excluded as the laser was inadvertently switched off during recording in one (SID 003) and bright ambient light adversely affected detection of fluorescence in another (SID 065). 21 patients were
included in the training stage, 14 in the testing stage and 6 in the final stage (Table 2.1).

Figure 2.6  STROBE diagram of study recruitment
2.4.2 Key results

The training stage was used to find the approximate dose of methylene blue that would be required by systematically increasing the dose by 0.05mg/kg until fluorescence was reliably observed in the tissues (Figures 2.7-2.10). The change in dose was dependent on the results of the previous participants and aimed to take into account the suspected pathology (Table 2.2). The testing phase narrowed down the dose from a limited range (0.3-0.5mg/kg, Figures 2.11-2.13) to a single dose that was taken forward to the final stage (0.4mg/kg).

A total of 15 patients received 0.4 mg/kg of methylene blue (thyroid=12, parathyroid=3). The median of the times to onset of fluorescence, time to peak fluorescence, gland to ‘drape’ ratios and gland to muscle ratios at this dose are shown in Table 2.3.
Table 2.2  Participant demographics, dose data and histological diagnosis

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<th>Group</th>
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<th>Dose 2nd Bolus mg/kg</th>
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<th>Histological Diagnosis</th>
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Figure 2.7  MB Fluorescence at 0.1mg/kg in Graves’ disease

A) Fluorescence from the central neck structures over time in a patient with Graves’ disease and administration of 0.1 mg/kg bolus of methylene blue. B) NIRF auto-fluorescence image taken at time point x demonstrating the thyroid (retracted by the surgeon’s fingers) and a normal parathyroid gland (arrow). C) NIRF image taken at time point y after administration of 0.1 mg/kg methylene blue in a patient with thyroid pathology.
Figure 2.8  MB Fluorescence at 0.1mg/kg in PHPT

A) Fluorescence from the central neck structures over time in a patient with PHPT and administration of 0.1 mg/kg bolus of methylene blue. B) NIRF auto-fluorescence image taken at time point x demonstrating the normal thyroid (retracted by the surgeon’s fingers), the enlarged parathyroid gland (1) and a normal parathyroid gland (2). C) NIRF image taken at time point y after administration of 0.1 mg/kg methylene blue in a patient with thyroid pathology.
Figure 2.9  MB Fluorescence at 0.2mg/kg in Graves’ disease

A) Fluorescence from the central neck structures over time in a patient with Graves’ disease and administration of 0.2 mg/kg bolus of methylene blue. B) NIRF auto-fluorescence image taken at time point x demonstrating the thyroid (retracted by the surgeon’s fingers) and a normal parathyroid gland (arrow). C) NIRF image taken at time point y after administration of 0.2 mg/kg methylene blue in a patient with thyroid pathology.
Figure 2.10 MB Fluorescence at 0.2mg/kg in PHPT

A) Fluorescence from the central neck structures over time in a patient with PHPT and administration of 0.2 mg/kg bolus of methylene blue. B) NIRF auto-fluorescence image taken at time point x demonstrating the normal thyroid (retracted by the surgeon’s fingers) and an enlarged parathyroid gland (arrow). C) NIRF image taken at time point y after administration of 0.2 mg/kg methylene blue in a patient with thyroid pathology.
Figure 2.1  MB Fluorescence at 0.3mg/kg in a multinodular goitre

A) Fluorescence from the central neck structures over time in a patient with multinodular goitre and administration of 0.3 mg/kg bolus of methylene blue. B) NIRF auto-fluorescence image taken at time point x demonstrating the thyroid (retracted by the surgeon’s fingers) and a normal parathyroid gland (arrow). C) NIRF image taken at time point y after administration of 0.3 mg/kg methylene blue in a patient with thyroid pathology.
Figure 2.12 MB Fluorescence at 0.3mg/kg in PHPT

A) Fluorescence from the central neck structures over time in a patient with PHPT and administration of 0.3 mg/kg bolus of methylene blue. B) NIRF auto-fluorescence image taken at time point x demonstrating the normal thyroid (retracted by the surgeon’s fingers) and an enlarged parathyroid gland (arrow). C) NIRF image taken at time point y after administration of 0.3 mg/kg methylene blue in a patient with thyroid pathology.
Figure 2.13  MB Fluorescence at 0.5mg/kg in Graves’ disease

A) Fluorescence from the central neck structures over time in a patient with multinodular goitre and administration of 0.5 mg/kg bolus of methylene blue. B) NIRF auto-fluorescence image taken at time point x demonstrating the thyroid (retracted by the surgeon’s fingers) and a normal parathyroid gland (arrow). C) NIRF image taken at time point y after administration of 0.5 mg/kg methylene blue in a patient with thyroid pathology.
Figure 2.14 Dose dependent MB fluorescence in Graves’ disease

Mean fluorescence of normal parathyroid and abnormal thyroid glands over at four different concentrations of MB in individuals with Graves’ disease
Figure 2.15  Dose dependent MB fluorescence in PHPT

Mean fluorescence of abnormal parathyroid and normal thyroid glands over time at four different concentrations of MB in individuals with primary hyperparathyroidism

Parathyroid  Thyroid
Table 2.3  Fluorescent patterns of thyroid and parathyroid glands at dose of 0.4 mg/kg body weight.

<table>
<thead>
<tr>
<th></th>
<th>Parathyroid gland (n=15)</th>
<th>Thyroid gland (n=14)</th>
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<td>Median time in seconds to onset of fluorescence (range)</td>
<td>22.0 (13-33)</td>
<td>23.0 (12-34)</td>
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<tr>
<td>Median time in seconds to peak fluorescence (range)</td>
<td>40.0 (26-88)</td>
<td>41.5 (27-103)</td>
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<tr>
<td>Median peak gland to drape ratio (range)</td>
<td>17.8 (6.7-54.8)</td>
<td>10.4 (5.7-29.7)</td>
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<td>Median peak gland to muscle ratio (range)</td>
<td>4.3 (1.7-6.8)</td>
<td>2.6 (1.1-5.1)</td>
</tr>
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</table>

2.4.3 Side Effects

There was a consistent, transient and spurious fall in oxygen saturation (pseudohypoxia) within the first minute of administration of methylene blue, which recovered completely in all patients within 30 seconds. Oxygen saturations were shown to fall briefly to as low as 65%. All other hemodynamic parameters were stable at this time. This was due to an artefactual change in light absorbance as the dye in plasma interferes with photometric measurement of blood oxygen saturation (Sidi, Paulus et al. 1987, Varon, Anderson et al. 1989). No patients had significant hypotension or ECG changes in relation to the administration of methylene blue. No visible staining was observed of the glands or the rest of the surgical field. Post-operatively, some patients commented on a transient discolouration of urine, but no other side effects were noted.

2.4.4 Clinical Outcomes

Six patients had hypocalcaemia on their first post-operative blood test (adjusted calcium 1.96 – 2.09 mmol/L). All six patients had undergone a total thyroidectomy. Four patients were started on sandocal, and two patients had sandocal and alfalcacidol. Five of these patients were asymptomatic and were discharged home to
return for further blood tests. One patient was readmitted as an emergency with symptoms of hypocalcaemia including pins and needles and right hand spasm. This patient was commenced on IV calcium gluconate and discharged home with sandocal and alfacalcidol. A further three patients were commenced on sandocal +/- alfacalcidol post-operatively. These patients had undergone parathyroidectomy and their post-operative calcium levels were within the normal range (adjusted calcium 2.36-2.50 mmol/L). It is presumed their post-operative PTH level was low as this is in keeping with the treatment protocol, but this was not recorded as part of the study.

2.5 Discussion

To our knowledge, this is the first phase I intra-operative dose response study of any fluorescent agent in the surgical literature. 0.05mg/kg was administered as the initial dose in both the thyroid and parathyroid groups. Fluorescence was visible in the thyroid, parathyroid gland and surrounding soft tissue even at this low dose but only lasted less than 10 seconds before returning to base line. This would be of limited use clinically as the fluorescence could be easily missed.

The dose of methylene blue was increased systematically, and the intensity and duration of fluorescence increased. At low doses (0.05mg/kg-0.2mg/kg, Figure 2.7 – 2.10) the fluorescence was not very bright and was difficult to discern, but the thyroid and parathyroid glands appeared to initiate fluorescence at the same time as each other and wash out at approximately the same rate. A reliable and predictable peak in fluorescence was visualised within seconds of the bolus being administered. As the dose increased towards 0.4mg/kg the parathyroid tissue appeared to fluoresce more intensely than the thyroid in the majority of cases. 0.4mg/kg methylene blue appeared to be the optimum dose at which both thyroid and parathyroid glands reliably fluoresced for a clinically useful period of time (Figures 2.14 and 2.15). Increasing the dose to 0.5mg/kg (Figure 2.13) did not improve the fluorescence seen nor the quality of images. At this dose however, parathyroid glands appeared to fluoresce brighter than the adjacent thyroid tissue in the majority of patients. This was in contrast to the results obtained in the animal model where the parathyroid glands showed a lower level of fluorescence and the fluorescence
washed out of the parathyroid glands faster than the thyroid (Antakia, Gayet et al. 2014). For identification and localisation of parathyroid glands, higher fluorescence in the parathyroid glands compared to surrounding soft tissue and thyroid it will make for easier identification. This is a more useful property compared to washout and is potentially beneficial for the clinical use of the technology.

Enlarged parathyroid glands (adenomatous or hyperplastic tissue in the context of primary hyperparathyroidism) showed increased fluorescence compared to normal glands. In one patient (SID 018) where an abnormal and normal parathyroid gland were both in view; at a dose of 0.1 mg/kg methylene blue, the enlarged parathyroid gland demonstrated a peak fluorescence parathyroid: muscle ratio of 4.5 compared to 2.1 for the normal appearing gland. With increasing dose of methylene blue, enlarged parathyroid glands showed intense fluorescence compared to surrounding tissues, and in comparison, to thyroid and normal parathyroid glands.

Eleven parathyroid glands thought to be abnormal by the surgeon, were sent for histology. All of these were shown on histology to be parathyroid tissue. Ten of these glands were abnormal (8 adenoma, 2 hyperplastic) and one was a normal parathyroid. As only glands considered abnormal at surgery could be removed and sent for histology, it is not possible to assess the accuracy of tissue identification. These results however were reassuring in that the surgeons correctly identified all 11 glands sent to pathology as parathyroids.

At the end of the testing phase, a dose of 0.4 mg/kg was adopted as the optimum dose. At this dose, fluorescence from normal parathyroid glands was deemed to be reliable.

At this dose, the thyroid and parathyroid glands showed a similar time to onset of fluorescence (p=0.109) regardless of pathology. The time to peak fluorescence was 41.5s (28-103) and 40s (26-88) for thyroid and parathyroid glands respectively and this was not significantly different (p=0.859). Parathyroid glands fluoresce more intensely than the thyroid when comparing the gland either to the drape (p=0.019) or to muscle (p=0.013). Abnormal parathyroid glands appear to fluoresce brighter than
normal parathyroid glands, but the numbers within these subgroups were too small to test for statistical significance.

Parathyroid glands have been shown to auto-fluoresce in the near infrared spectrum. Two other studies evaluating parathyroid auto-fluorescence at wavelengths 750 nm (De Leeuw, Breuskin et al. 2016) and 785 nm (Paras, Keller et al. 2011, McWade, Paras et al. 2014) have shown that the parathyroid fluoresces between 2 to 11 times more than the thyroid or other neck structures (Paras, Keller et al. 2011, McWade, Paras et al. 2014, De Leeuw, Breuskin et al. 2016). The mechanism behind parathyroid auto-fluorescence is not yet understood and the fluorescence appears to be independent of pathology and gland viability (McWade, Paras et al. 2013, De Leeuw, Breuskin et al. 2016).

This study has demonstrated auto-fluorescence at an excitation wavelength of 680 nm, and this has not previously been observed (Figure 2.1). The parathyroid glands were often noted to be fluorescent and distinct from surrounding soft tissue prior to administration of methylene blue (auto-fluorescence). The mean difference in relative intensity between the PG and thyroid gland was +1.3 (n=14) in the 10 seconds prior to methylene blue administration (calculated only in patients administered 0.4mg/kg).

Although auto-fluorescence may aid intra-operative parathyroid identification, a further assessment of viability is needed to help make the decision for autotransplantation as auto-fluorescence appears to be independent of perfusion. This is highlighted by an example in this study where there was concern about viability in one parathyroid (028). Here, the PG was visible with use of the Fluobeam® 700 prior to the bolus of methylene blue. The PG did not show any change in fluorescence after the bolus for up to 7 minutes after injection, which was not in keeping with other glands that were deemed to be viable. The demonstration of auto-fluorescence at 680 nm that is also compatible with methylene blue fluorescence is of significance because the same device/technology can be used to both identify parathyroid glands and also determine their viability after methylene blue administration, but methylene
blue may not be required in all patients and could be reserved for when there are concerns with viability.

Alternative imaging systems such as FLARE™ and Mini-FLARE™ have been used in some studies to assess methylene blue fluorescence of parathyroid adenomas (van der Vorst, Schaafsma et al. 2014, Tummers, Schepers et al. 2015). A fixed dose of 0.5 mg/kg was used in all patients included in the studies, which concluded that parathyroid adenomas could be localised intra-operatively using this method and that normal Parathyroid glands could occasionally be seen (Tummers, Schepers et al. 2015). However, the basis of this fixed dose is not clear, and no dose response assessments were included in the published reports.

Our study has shown that both normal and abnormal parathyroid glands can be identified at doses as low as 0.4 mg/kg MB (around 7.5 – 17.5 times lower than doses previously used for macroscopic blue staining). At this dose, the viability of a parathyroid gland can be confirmed, and it may be possible to differentiate parathyroid adenoma from normal glands during a neck exploration. Methylene blue

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**Figure 2.16  Parathyroid auto-fluorescence and MB fluorescence**

*Patient with thyroid nodule undergoing right hemithyroidectomy. A) Colour photograph of surgical field. Thyroid retracted medially by surgeon’s fingers. Normal superior parathyroid gland identified dotted circle. B) NIRF image prior to injection of methylene blue showing parathyroid auto fluorescence (dotted circle). C) NIRF image at peak parathyroid fluorescence (42 seconds after injection of 0.4mg/kg methylene blue) showing significant parathyroid gland fluorescence (dotted circle) compared to thyroid and surround soft tissues.*
fluorescence can also be used to differentiate between parathyroid gland and surrounding soft tissue such as lobules of brown fat or lymph nodes. Although the thyroid gland fluoresces following methylene blue administration, the differences in auto-fluorescence along with methylene blue fluorescence patterns, assist in distinguishing parathyroids from thyroid tissue.

This study shows the potential for parathyroid auto-fluorescence at 680 nm to be used as a screening method for early parathyroid identification and methylene blue fluorescence following intravenous administration for confirmation of parathyroid glands and assessment of its viability. This may be of help in a number of different scenarios – early parathyroid identification to enable preservation of normal glands during thyroidectomy; assessment of viability of parathyroid glands at the end of thyroidectomy to determine the need for auto-transplantation; and early identification of enlarged parathyroid glands in parathyroid surgery. This will in turn improve outcomes of thyroid and parathyroid surgery. As the onset of fluorescence from the tissues is seen within seconds of methylene blue administration this method enables a rapid assessment of viability without extensive preparation.

As discussed in the animal model study, we found that visual differentiation of tissues was subjectively easier in real time than when analysing the images after the procedure. A number of factors may have affected this. It is easier to correlate the 2D image on the screens with the intra-operative field in real time. The contrast and details on the screen appeared more defined than the captured images. The captured images may be of lower resolution due to file compression or be affected by movement as they only capture one image a second so there is potential for the image to be blurred if there is movement. There will always be small amounts of movement with patient respiration even if the camera is fixed and the surgeons keep retractors still. Files were downloaded as tiff files which, in theory, should not be compressed but within the software some compression of the image may occur prior to download as high-resolution images use a lot of data space and therefore lower resolution images may be used. This may affect the data gathered to provide an objective assessment of fluorescence using Image J. There are a number of factors that may affect the fluorescence intensity. These include the distance of the camera
from the tissues due to operator use or depth of the surgical field, the position of the gland within the field of view and degradation of the image due to the original sterile cover. The camera works best at a working distance of 20cm. The camera was held at this approximate distance throughout recording, but the surgical field is not flat, therefore there may be parts which are closer or further away than the optimal distance, which may affect fluorescence intensity.

The camera also has an optimum region in the centre of the field of view where the lasers are focused. Tissue in the centre of the field will show more intense fluorescence than tissue in the peripheries even if they are the same tissue. Therefore, the position of the glands within the view may affect how intensely they appear to fluoresce. In this study, to get readings from both the thyroid and parathyroid the camera was orientated to get both glands within the view. This may affect the fluorescence detected by the camera from the more peripheral areas of the field of view compared to the centre as the laser excitation is highest in the centre. The angle at which the camera is directed at the tissue is affected by many factors including the location of the gland and the depth of the neck. A stand or clamp to fix the camera a pre-determined distance from the operating table was suggested in the design of the study, however one was not already available and would be costly to manufacture. In practice this was also unfeasible as patients’ neck vary significantly from patient to patient and therefore the position required for one patient would not be the same as the next.

It was also noted that over the period of 10 minutes there was operator fatigue and increased movement of the tissues. During the repositioning of the instruments or surgeon’s fingers used for retraction of the thyroid, there were changes in fluorescence due to changes in exposure, movement from the centre of the field of view or changes in blood supply due to pressure on adjacent vasculature. This resulted in anomalous peaks in fluorescence. Whilst attempts were made to take the reading from muscle, some of these readings may have been at least in part been from adjacent subcutaneous soft tissue as it was difficult to always ensure that the strap muscles were in view during recording. Ideally, the tissues and camera should have been maintained in position with fixed retractors and clamps. However, these
do not exist in practice and would be expensive to commission and take time to adjust in theatre. Also, the thyroid is extremely delicate, and surgeons do not routinely use instruments to retract the gland so there would be concern of injury to the delicate structures.

The methylene blue was injected via a tubing connected to a peripheral cannula. The size of this tubing varied in diameter, depending on the anaesthetist’s preference. It was noted that despite flushing of the tubing after administration of methylene blue, occasionally, methylene blue was left in the tubing. It is therefore recommended that methylene blue is administered via a fine tube or connector to the peripheral cannula.

The standard surgical drapes used at STHNFT including the towels placed to protect the incision are coloured blue. It was found during this study that the fluorescence from the drapes was higher than other surfaces. This was used as a baseline to compare the changes in fluorescence over time. Although the fluorescence of the drape did not change over time, it did differ with changes in the ambient lighting in theatre. There is no clear evidence that the colour of the surgical drapes can have a difference on the fluorescence. However, anecdotal evidence from two companies (Fluoptics and Medtronic) who produce near infrared fluorescence imaging systems suggest that the blue drapes can interfere with subtle changes in fluorescence. This could be important when using parathyroid auto-fluorescence and an alternative colour, such as green, should be used to avoid this issue.

2.6 Conclusion

In conclusion, parathyroid glands can be identified intra-operatively using NIRF imaging following administration of intravenous methylene blue doses as low as 0.4 mg/kg and parathyroid fluorescence is observed within 60 seconds of administration. Parathyroid glands fluoresce more intensely than the thyroid tissue. Enlarged parathyroid glands are distinguished from normal glands based on the fluorescence intensity. This method has potential in enabling early and accurate identification of parathyroid glands and determining gland viability; thereby improving outcomes in thyroid and parathyroid surgery. A Phase II study of this
protocol to evaluate clinical outcomes such as post thyroidectomy hypoparathyroidism is now required. We have also shown that a study investigating at parathyroid auto-fluorescence would be of clinical benefit and is feasible with this technology. This has now been designed and had funding approved and is described in chapter 6.4. The design and approval of the phase 2 study was undertaken after the results of this study was published and the time required to complete the approval process and a delay in starting the study due to the COVID-19 pandemic meant the study was not commenced with time to include it in this thesis.

Fluoptics™ have used the feedback from this study and from other users of the Fluobeam 700® to make crucial changes to the design of the newest model, Fluobeam®LX. The sterile cover has now been redesigned to incorporate a clear plastic cap which fits over the entire end of the camera, including the LEDS and laser. At the start of the NIRF study conducted in this thesis, the sterile cover had a small clear disc which was lined up with the camera but did not cover the LEDs and laser. This affected the peripheral vision of the camera and degraded the image at the edge of the screen. It affected the quality of the images seen by the operating surgeon. This was fed back to the company at the start of the NIRF study and a temporary fix was made which improved the images and the new cover was manufactured to improve the image quality and the ease of intra-operative application of the cover.

The second change that has been made is to the shape of the camera. The Fluobeam®LX now features a more ergonomic design with an easier to hold shape and a joystick that allows the surgeon to control the function of the camera from the operating table. The greatest change to be addressed, and giving the camera its name, is the camera’s sensitivity to ambient light. The new camera does not require the ambient light to be altered, but it is recommended that the bright, overhead operating lights are switched off or turned away from the operating field. This allows for less disruption to the operation and a safer working environment for theatre staff. The Fluobeam®LX also comes with its own screen for superior image projection and easier cleaning for infection control. The field of depth has been increased to 5cm which will reduce the possibility of small glands being overlooked by the surgeon. There is a difference in the excitation wavelengths used in the Fluobeam 700® compared to the Fluobeam®LX. The Fluobeam 700® used
excitation wavelengths of 680nm and collected wavelengths over 700nm whereas the Fluobeam®LX has an excitation wavelength of 750nm and collects wavelengths over 800-900nm. This is to specifically aid detection of parathyroid autofluorescence and ICG can be used as an adjunct to assess parathyroid gland perfusion.

This change in interest in the field to parathyroid auto fluorescence and a reluctance to use methylene blue given its exclusion criteria has meant that manufacturers have made new technologies compatible with ICG rather than methylene blue. The Phase 2 trial designed and described in chapter 6.4 originally proposed to use methylene blue as in this study but the change in available technology and the increasing interest in ICG led to a change in the protocol of the study and methylene blue has been usurped by ICG.
Chapter 3: Electrical Impedance Spectroscopy in Thyroid and Parathyroid Surgery – a ‘proof of concept’ phase I human study
3.1 Background to the study

Electrical Impedance Spectroscopy (EIS) measures the resistance to an electrical circuit over a range of frequencies. Impedance is the combination of resistance and reactance (both inductive and capacitive). When tissue is placed as part of the circuit the impedance of that tissue can be measured giving a spectrum specific to that tissue. As described in chapter 1.6.3, difference in the tissues produces difference spectra and EIS can detect differences in the morphology of a biological tissue due to the change in the shape and structure of the cells within the tissue (Brown, Tidy et al. 2000, Brown, Milnes et al. 2005, Tidy, Brown et al. 2013). This therefore has the potential to differentiate between two tissues if their underlying cellular structure is different.

EIS is currently used as an adjunct to colposcopy to determine abnormal areas in the cervical epithelium that may be subject to a targeted biopsy. This has shown to increase the accuracy of detection of high-grade disease. The positive predictive value increased from 78.1% (95% CI 67.5–86.4) to 91.5%. Specificity was also increased from 83.5% (95% CI 75.2–89.9) to 95.4%, but sensitivity was significantly reduced from 73.6% (95% CI 63.0–82.5) to 62.1%, and the negative predictive value was unchanged (Tidy, Brown et al. 2013). Impedance differences between tissues such as the thyroid and parathyroid could be exploited to help surgeons in the intra-operative identification of parathyroid glands. Just as EIS of the cervix gives real time feedback to a colposcopist of whether an area is abnormal and a biopsy is required (Tidy, Brown et al. 2013), EIS may give real time feedback to the surgeon during dissection of the central neck compartment. Early confirmation that tissue is indeed a parathyroid gland would avoid damage to the tissue. It would particularly be of use in patients undergoing redo surgery where the anatomy may be difficult to clarify or if central compartment lymphadenectomy was indicated.

This hypothesis was tested in a “proof of concept” study using a rabbit model (Antakia, Brown et al. 2016). The aim of this study was to investigate whether soft tissues in the neck could be differentiated using EIS. A rabbit model was chosen in view of the presence of two extra-thyroidal parathyroid glands in rabbits that are
large enough to be able to take readings using an existing EIS device (APX100 manufactured by Zilico™). EIS readings over 14 frequencies were taken from the thyroid, external parathyroid glands and strap muscle of nine freshly culled New Zealand White rabbits using the APX100 device. The rabbits were euthanised as part of an unrelated research project and after culling, the rabbits were kept on heat pads to simulate in vivo temperature conditions. Both in vivo and ex vivo readings were taken from the glands identified by visual inspection. An EIS spectrum from each tissue was produced. The excised tissue’s identity was confirmed on histology. The results of this study showed that at low frequencies the impedance of thyroid tissue was significantly higher than parathyroid tissue (Figure 3.1). The electrical impedance (EI) of ex vivo tissue was significantly higher than the corresponding in vivo readings (p <0.001; Wilcoxon signed rank test) (Antakia, Brown et al. 2016). This is likely to be due to the drop in temperature; impedance rises by approximately 2% per degree centigrade (Edd, Horowitz et al. 2005).

![Median in vivo impedance of thyroid, parathyroid and muscle](image)

**Figure 3.1   EIS in rabbits**

*Median electrical impedance spectra of New Zealand rabbit thyroid gland, parathyroid gland and muscle over 14 frequencies (Antakia, Brown et al. 2016)*
The ratio of low (152Hz) to high (312kHz) frequency of *in situ* thyroid, parathyroid and muscle was compared and found to be significantly different (p <0.001; Friedman’s 2-way analysis of variance by ranks test) (Antakia, Brown *et al.* 2016). A box and whisker plot of the ratios shows clear separation of the *in vivo* thyroid and parathyroid tissues (Figure 3.2).

**Figure 3.2**  Comparison of *in situ* EIS in thyroid and parathyroid glands in rabbits

*Box and whisker plot comparing the ratio of *in situ* electrical impedance at 2 frequencies (152 Hz and 312 kHz) in thyroid, parathyroid and muscle of New Zealand rabbit (Antakia, Brown *et al.* 2016).*
This study concluded that the device was able to produce EI spectra for thyroid and parathyroid glands. The thyroid and parathyroid spectra were easily separated from each other both in vivo and ex vivo. This study showed that EIS had the potential to differentiate between different tissues in thyroid and parathyroid surgery. The difference between in vivo and ex vivo EI spectra were also significantly different, and this observation indicates potential in identifying ischaemic parathyroid glands during neck surgery. The decision whether to auto-transplant an ischaemic gland is based on the surgeon’s judgement of viability. A technique that can accurately detect ‘loss of viability’ will lead to excision and re-implantation of non-viable glands and this may prevent long term hypoparathyroidism (Lo 2002, Athanasopoulos, Kyriazi et al. 2011, Ahmed, Aurangzeb et al. 2013). Reducing unnecessary auto-transplantation may also reduce short term post-operative hypocalcaemia (Edafe and Balasubramanian 2017).

To test this technology further, we have designed a clinical phase 1 feasibility trial to test this hypothesis in humans using an EIS device which is a modification of the APX100 device used in the rabbit study. The ZedScan™ system (Figure 3.3) manufactured by Zilico™ consists of a handheld, cordless, portable device and a docking station that downloads data to a laptop computer where the software is installed. A disposable, single use sensor cover is placed over the nose cone and tip of the hand-held unit. The tip of the sensor is 5.5 mm in diameter and has four gold electrodes that make contact with silver/silver chloride points mounted on a plate at the tip of the sensor cover. The sensor cover is placed in contact with the tissue. Coloured LED lights visible halfway down the nose of the device and a screen on the handset alert the operator to problems and indicate whether a measurement is ready to be taken (continuous green), being processed (continuous orange) or if the device has failed to take a measurement (flashing red).

The diameter and spacing of the four electrodes determine the flow of current across the tissue. The current (<12 μA point to point) is passed between one adjacent pair of electrodes and the impedance is measured between the second pair. Each reading is a series of measurements performed over 14 frequencies (ranging from 76 to 625000 Hz) in approximately 20 milliseconds. The handset stores up to 12
readings. The handset is docked, and data downloaded and transformed into a measure of mean EI and standard deviation (SD) at each frequency. The measurements across the frequencies can then be plotted to produce a spectrum.

Figure 3.3  ZedScanTM portable handset and docking station
The user screen is visible on the handpiece and LED lights are halfway down the nose cone (www.zilico.co.uk).
3.2 Aims

The aim of this study was to evaluate the potential for EIS to be used in human thyroid and parathyroid surgery. Specific objectives were:

1. To determine EI spectra of normal and abnormal human thyroid, parathyroid, lymph nodes, fatty tissue and skeletal muscle.

2. To compare the EI patterns of the parathyroid with the other soft tissue structures in the human neck.

3. To investigate changes in EI before and immediately after excision of soft tissue structures in thyroid and parathyroid surgery.

4. To develop a protocol for the use of EI in the accurate differentiation of parathyroid glands from other structures including thyroid nodules, adipose tissue and lymph nodes.

3.3 Methods

3.3.1 Study Design

This Phase 1 feasibility study in humans was designed as a prospective cohort, single arm study. It was conducted at a single centre (Sheffield Teaching Hospitals NHS Foundation Trust) and included patients under the care of two Consultant Endocrine Surgeons. The population were patients with benign or malignant thyroid and parathyroid conditions undergoing surgical management. Patients who were undergoing hemi thyroidectomy, total thyroidectomy or bilateral, unilateral or targeted neck explorations were included. During surgery, which was conducted as routine, EIS readings from the tissues in the neck were taken. The EIS results were not available to the operating surgeon and therefore had no impact on intra-operative decision making. No comparator was used in this study. The primary outcome of this study was the identification of parathyroid glands. Secondary outcomes were differences in the EIS spectra between normal and abnormal parathyroid glands.
The ZedScan™ device and consumables were on loan from Zilico for use in this study. They provided technical support where required. Professor Brian Brown, emeritus Professor of the University of Sheffield and founding member of Zilico, provided expert opinion, data monitoring and statistical guidance in the analysis of the data and this input is documented in this thesis. Dr Peter Highfield, Technical Director at Zilico, monitored data for quality to ensure the raw data sets were recorded correctly. No conflicts of interest are declared.

3.3.2 Ethical Approval

The study protocol and participant documents were reviewed by members of Parathyroid UK (a UK based national charity for patients with hypoparathyroidism) for suitability, clarity and lay understanding (Appendix 3.2, 3.4 and 3.5). The study and research documents were approved by the Regional Research Ethics Committee (IRAS 245001) (Appendix 3.1 and 3.3). The study was registered with the local STH Research and Development department (ref no. STH18736) and ClinicalTrials.gov (NCT02901873) (ClinicalTrials.gov 2016). Based on the current CE registration of the ZedScan™, an application was not required to the MHRA or recommended by the research ethics committee.

3.3.3 Modification of Zedscan™ to ensure safety and efficacy

The ZedScan™ device and single use covers are CE marked for use in humans. The single use sensor covers are manufactured in a clean environment and for use in colposcopy. However, they are not required to be sterilised and this was an impediment for their use in the operating theatre. To perform EI measurements with ZedScan™ in human surgery, a series of experiments were conducted in three stages (by Synergy Health) to determine the appropriate sterilisation procedure for the sensor tips both to minimise risk of infection and to enable an effective measurement of impedance. In stage one, boxes of the sensor tips were subjected to different levels of gamma irradiation (15-45 kGy) and samples from each box were assessed for physical appearance, mechanical properties and electrical performance. These tests demonstrated that the physical properties of the sensor tips were not significantly altered following high dose radiation. Stage two determined the
minimum dose required to sterilise the sensor covers as 25 kGy. Sensor covers were irradiated and then tested for bioburden according to the relevant standards. Stage three prepared the final batch of sensor covers ready for use. A small number of these sensors were tested again to ensure function and sterility prior to the start of the study.

3.3.4 Participant Eligibility

All adult patients (18 years and over) undergoing thyroid and/or parathyroid surgery at the Royal Hallamshire Hospital (Sheffield Teaching Hospitals NHS Foundation Trust, UK) in the Endocrine Surgery unit (General Surgery Directorate) were eligible to be included in the study.

Patients were excluded if they were undergoing thyroglossal cyst surgery or re-do procedures only involving resection of lymph nodes. Patients were also excluded if they were unable to understand spoken and written English, unable to give adequate informed consent or if they had a positive pre-operative pregnancy test.

3.3.5 Participant Recruitment

Patients attending the outpatient department between July 2016 and March 2017 at Sheffield Teaching Hospitals who were placed on the waiting list for a thyroid and/or parathyroid operation for either benign or malignant conditions were screened for eligibility. During the clinic consultation for surgery, the study was discussed with the patient. If the patient was interested in participating, they were given a participant information sheet (Appendix 3.4) in clinic, or this was posted to their address. If the research team was available on the date of the proposed operation, the eligibility criteria were reviewed, and the patients were approached in the Theatre Admissions Unit or on the ward before their operation. The study was explained again in detail and the patient was given opportunity to ask questions and clarify any concerns with the research team. Following which informed, written consent was gained.
3.3.6 Researcher Training

A training day for the research team was held to familiarise the operative team to the equipment. The equipment was demonstrated by Dr. Peter Highfield, Technical Director of Zilico™ and was attended by the research team including the surgeons, a representative from the Research and Development department and a senior operating department practitioner.

The technique to keep equipment sterile when passing the probe into the sterile surgical field was initially determined. An open ended sterile, transparent sheath would be attached to the sterile sensor cover with an adhesive strip. The probe would be placed inside the sensor cover, assuring contact by confirmation on the device screen. The plastic sheath would then be passed over the handpiece and secured at the end by tying the sheath. Thus ensuring that the entire handpiece was enclosed in a sterile covering. The screen and buttons on the handpiece could be visualised through the sheath and allowed the surgeon to activate the handpiece. The team familiarised themselves with the equipment and practised assembling the device. As the device is cordless it has a limited battery supply and is charged when docked. It was agreed that the device would only be placed into the sterile covering once the surgeons were ready to take a reading, thus reducing time away from the docking station due to the variable time between the start of the operation to localising tissue for measurements.

The research team and surgeon were familiarised with the software and how this would correlate with the data collection form approved by the Research Ethics Committee (Appendix 3.6). The research team were familiarised with the data output to allow interpretation of results.

All members of the research group held valid Good Clinical Practice (GCP) certificates.
3.3.7 Intra-operative Methods

At surgery, the central compartment of the neck was opened, and dissection of the tissues commenced as per standard practice. When the surgeon was satisfied they had identified the thyroid and parathyroid gland(s) on one side of a neck, the device was placed within the sensor cover by the research team and contact between the electrodes and the plate in the tip of the sensor cover was indicated with a confirmation screen on the device and cessation of the audible alarm and lights on the nose cone. The handpiece was then covered over with the sterile plastic sheath, tied off and passed to the surgical team. If the patient was undergoing a bilateral operation, readings were taken from the first side before moving on to dissection of the second side. This was to reduce the risk of unnecessary dissection to expose glands a second time and the devascularisation of either the thyroid or parathyroid glands. In two cases no parathyroid glands were confidently identified by the surgeon after employing all usual techniques. In these cases, readings were taken from all the other identified structures as it was felt further dissection to identify the parathyroid glands could lead to increased morbidity including hypoparathyroidism or recurrent laryngeal nerve injury; this is in line with standard surgical procedure for patients not participating in a study.

Readings were taken from the neck depending on the type of the operation in accordance with the data collection form. The device was able to hold up to 12 readings which was identified by the data collection tool. If fewer than 12 readings were taken, additional repeated readings from the glands were taken. In taking repeat readings, parathyroid tissue was prioritised over thyroid tissue and abnormal parathyroid tissue was prioritised over normal parathyroid tissue. The screen on the handset presented the readings out as a clock face, each position corresponding to a reading documented on the data collection form (Figure 3.4B and Appendix 3.6).

For each reading, the tip of the probe was placed in contact with the tissue of interest (Figure 3.5). Care was taken not to place too much pressure on the tissue to avoid damage to the gland. When the probe had established homogenous contact with the tissue, the indicator light turned green to signal it was ready to take a
reading (Figure 3.6) or orange to signal it had failed the quality assurance (QA) check. The screen on the handset also indicated this by the colour in the top right-hand corner changing from amber to green and displaying "Point Failed QA" to "Point Passed QA" (Figure 3.4C).

The surgeon initiated the reading by pressing the central button on the device console (Figure 3.5). The device was kept still and in contact with the tissue whilst the reading was taken. The indicator light turned orange whilst a reading was being taken (2-10 seconds) (Figure 3.7), and completion of the reading was followed by a double beep. If the reading failed, then a two-tone beep sounded, and the indicator lights flashed alternate green and orange. A confirmation screen that the reading failed had to be acknowledged before another reading was taken to ensure that the surgeon did not move onto the next reading without being aware of the failure to take reading (Figure 3.4D).
A) ZedScan™ patient details confirmation screen.

B) ZedScan™ device screen. Each reading is indicated by a circle on the clock face. Green circles indicate a successful reading. Blue circle indicates the current reading which turns yellow during a measurement. White circles denote empty slots.

C) ZedScan™ QC checks. Change in amber to green in the top right hand corner and banner across the screen indicate the device is ready to take a reading.

D) ZedScan™ reading failed warning screen.

**Figure 3.4: ZedScan™ screen explanations**
Figure 3.5 Surgeon using ZedScanTM intra-operatively with sterile cover over the handset
Figure 3.6  Green LED on the nose cone indicating the device is ready to take a reading

Figure 3.7  Orange LED on the nose cone during a measurement
The researcher, who was unscrubbed in theatre, documented in real time where readings were successfully taken from on the data collection form (Appendix 3.6). Any problems taking readings from specific tissues was documented. Other recorded findings included tissue type and other specific gland characteristics (e.g., abnormal, bruised, and encased in fat) that may alter the interpretation of the reading. If the reading failed on any given point, three attempts were made to take a reading from that tissue and if all three readings failed the surgeon moved on to a different tissue and the failed reading was documented. The patient’s temperature from a nasal temperature probe was also documented.

Glands that were abnormal were resected in accordance with standard surgical practice. In one case, a normal parathyroid gland that was inadvertently devascularised was also resected and then auto transplanted. Ex vivo glands had their temperature recorded using an infrared thermometer to reduce contact with the tissues and then EI readings were taken prior to the tissue being placed in formalin and sent to histopathology.

Following the recordings, the device was handed to the researcher, ‘docked’ and linked to a laptop. Anonymised participant details including study ID and date of surgery were entered into the software on the laptop. Once the device was lifted from the dock, participant details were visible on the screen and confirmed with the research documents (Figure 3.4A).

The device would try and analyse the data using the colposcopy software and indicated that biopsies should be taken, which was not relevant to this study. The raw data was extracted from the software onto a Microsoft Excel spreadsheet.

After surgery, patients were managed in accordance with standard clinical practice and appropriate follow up arrangements were made. The study protocol did not influence any aspect of pre-operative or post-operative care. Histology results from the tissues resected were collected from the hospital reporting system for correlation with the data collected.
3.3.8 Internal Quality Control Checks

In the normal working of the software there are two layers of quality control (QC) checks. The first is that described previously, when the probe comes in contact with a tissue. This QC check ensures the probe has homogenous contact with tissue. This QC check remained in place for this study.

The second QC check does a rapid internal analysis of the readings and rejects any readings that fall outside of what is deemed acceptable i.e., what would be an 'anomalous' result for the cervical tissue. This QC check was removed at the start of the study, so the device 'accepted' any reading taken. This was done to collect as much information as possible without using restrictions identified for the cervix. This meant that there were very few rejected readings. However, when the data was evaluated by the research team there were frequent ‘impossible’ readings (negative values, rising impedance with frequency) which had to be excluded. As the data could not be evaluated until after the handset was docked and the data downloaded; repeat measurements from the tissues of interest at the time of surgery were not possible as the surgery would have to continue. This issue was discussed by the research team and a consensus was reached to reinstate the second layer of QC checks. The main objective of this step was to improve the quality of data collected and reduce the relative proportion of excluded readings. This was implemented from patient 30 onwards.

3.3.9 Sample size and data analysis

Sample size estimations were not performed as this was a pilot study and there was no published data on electrical impedance in human thyroid and parathyroid tissue. Measurements were taken from both normal glands and abnormal glands; the latter may be associated with a number of different benign and malignant pathologies. As EI may differ based on pathology, it was considered reasonable to include a number of patients for each of the different thyroid and parathyroid pathologies commonly encountered (colloid goitre, thyroid cancer, Graves’ disease, parathyroid adenomas and hyperplasia). On this basis, a proposed a priori sample size of around 50 patients with an approximately even distribution between those with thyroid and parathyroid
disease was considered appropriate. On this basis, around 25 patients in each group (thyroid and parathyroid surgery) were recruited.

EI spectra raw data was downloaded to a Microsoft Excel database and the relevant information (mean real impedance, standard deviation of real impedance, mean imaginary impedance and standard deviation of imaginary impedance) was extracted. Analysis of the data was primarily descriptive based on observations made on the spectra plotted from the raw data. Inferential data analysis was performed in keeping with methods used in the rabbit model (Antakia, Brown et al. 2016) including Friedman’s 2-way analysis of variance by ranks test using SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) and ROC curves using GraphPad (GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com).

3.4 Results

3.4.1 Demographics

Eighty patients were screened for eligibility in the outpatient clinic and accepted a participant information sheet. The research team was not available on the day of surgery for 10 patients and a further 10 patients were not allocated a date for surgery by the end of the study. Sixty patients were approached for a second time to confirm eligibility and gain written consent. Four patients declined consent (Figure 3.8).

Fifty-six patients participated in the study (9 males, 47 females). The median age of participants was 53.5 (range 20-85). Thirty-nine participants had surgery for thyroid pathology; 21 for parathyroid pathology; and 4 had surgery for both thyroid and parathyroid pathology. Two patients (one from the thyroid and one from the parathyroid groups) were subsequently excluded from the study as the device failed intra-operatively by powering off and on again and deleting data in the process prior to docking. Following these incidents, the device was replaced with updated software to prevent any further data loss.
Twenty-four patients underwent hemi-thyroidectomy for a thyroid nodule. On histology, this was further characterised as adenoma (n=10), carcinoma (n=4), hyperplasia (n=2) and multinodular or colloid change (n=8). Nine patients underwent total thyroidectomy for Graves’ disease. Four patients had a total thyroidectomy for multinodular goitre. One patient had thyroidectomy for proven thyroid carcinoma and one patient underwent completion thyroid lobectomy.

A total of 28 ‘parathyroid glands’ were sent for histology. This did not include one patient who had inadvertent parathyroid gland excision whilst undergoing a thyroid lobectomy discovered on histology. Nineteen parathyroid adenomas were excised. Two of these patients also had a second parathyroid sent for assessment that was reported as normal tissue. Another of these patients had a second parathyroid gland sent for histology that was reported as thymus. Six parathyroid glands were sent from two patients, and these were reported as parathyroid hyperplasia.
Figure 3.8  STROBE diagram of recruitment for EIS study
Table 3.1  Indication for surgery and histological diagnosis

<table>
<thead>
<tr>
<th>Indication for surgery</th>
<th>Number</th>
<th>Histological Diagnosis</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid Nodule</td>
<td>24</td>
<td>Thyroid Adenoma</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thyroid Carcinoma</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thyroid Hyperplasia</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multinodular/Colloid</td>
<td>8</td>
</tr>
<tr>
<td>Graves’ Disease</td>
<td>9</td>
<td>Graves’ Disease</td>
<td>9</td>
</tr>
<tr>
<td>Multinodular Goitre</td>
<td>4</td>
<td>Multinodular Goitre</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Toxic Nodular Goitre</td>
<td>1</td>
</tr>
<tr>
<td>Thyroid Carcinoma</td>
<td>1</td>
<td>Thyroid Carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Completion Thyroidectomy</td>
<td>1</td>
<td>Follicular adenoma</td>
<td>1</td>
</tr>
<tr>
<td>Primary Hyperparathyroidism</td>
<td>21</td>
<td>Parathyroid Adenoma</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parathyroid Hyperplasia</td>
<td>2</td>
</tr>
</tbody>
</table>
3.4.2 EIS Data Analysis

Raw data from the intra-operative readings were independently analysed by the Sheffield research group and Professor Brian Brown, Emeritus Professor of Medical Physics at the University of Sheffield.

A total of 603 EIS spectra were downloaded. Spectra were reviewed initially for suitability. The spectra were rejected if negative values were recorded, or the spectra showed impedance rising with frequency. The cause of these phenomena is likely to have been poor electrode contact with the tissue. 184 spectra were rejected. The majority of rejected spectra were those taken from fat and muscle.

Readings taken before and after the second set of QC checks showed that the number of rejected readings reduced from 39.1% to 18.0%. The rate of rejection decreased in all tissue types after the QC check was implemented. Significantly fewer readings were obtained from fatty tissue after the second set of QCs were introduced. 30 readings from superficial fat were taken before the second QCs of which 20 (66.7%) were rejected. After the second QCs were applied, only 2 readings were obtained, one of which were rejected on later analysis (50%). Similarly, 27 readings were obtained from visceral fat, 15 (56%) of which were rejected before the second QC and 8 obtained after with 2 (25%) rejections.
Table 3.2  Total readings and rejections by tissue type

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Total readings taken</th>
<th>Total readings rejected</th>
<th>% readings rejected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal thyroid</td>
<td>55</td>
<td>8</td>
<td>14.5</td>
</tr>
<tr>
<td>Abnormal thyroid</td>
<td>72</td>
<td>5</td>
<td>6.9</td>
</tr>
<tr>
<td>Normal Parathyroid</td>
<td>124</td>
<td>37</td>
<td>29.8</td>
</tr>
<tr>
<td>Abnormal Parathyroid</td>
<td>43</td>
<td>8</td>
<td>18.6</td>
</tr>
<tr>
<td>Superficial Fat</td>
<td>32</td>
<td>21</td>
<td>65.6</td>
</tr>
<tr>
<td>Visceral Fat</td>
<td>35</td>
<td>17</td>
<td>48.6</td>
</tr>
<tr>
<td>Muscle</td>
<td>51</td>
<td>33</td>
<td>64.7</td>
</tr>
<tr>
<td><em>Ex vivo</em> Thyroid</td>
<td>128</td>
<td>32</td>
<td>25.0</td>
</tr>
<tr>
<td><em>Ex vivo</em> Parathyroid</td>
<td>54</td>
<td>21</td>
<td>28.9</td>
</tr>
<tr>
<td>Other</td>
<td>9</td>
<td>2</td>
<td>22.2</td>
</tr>
<tr>
<td>Total</td>
<td>603</td>
<td>184</td>
<td>30.5</td>
</tr>
</tbody>
</table>
Table 3.3  Number of readings taken and rejected before and after introduction of second layer of QC checks

<table>
<thead>
<tr>
<th></th>
<th>Before QC Checks applied</th>
<th></th>
<th>After QC Checks applied</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Readings Taken</td>
<td>Readings Rejected</td>
<td>% rejected</td>
<td>Readings Taken</td>
</tr>
<tr>
<td>Normal thyroid</td>
<td>28</td>
<td>8</td>
<td>28.6</td>
<td>27</td>
</tr>
<tr>
<td>Abnormal Thyroid</td>
<td>43</td>
<td>5</td>
<td>11.6</td>
<td>29</td>
</tr>
<tr>
<td>Normal Parathyroid</td>
<td>76</td>
<td>25</td>
<td>32.9</td>
<td>48</td>
</tr>
<tr>
<td>Abnormal Parathyroid</td>
<td>16</td>
<td>5</td>
<td>31.3</td>
<td>27</td>
</tr>
<tr>
<td>Superficial Fat</td>
<td>30</td>
<td>20</td>
<td>66.7</td>
<td>2</td>
</tr>
<tr>
<td>Visceral Fat</td>
<td>27</td>
<td>15</td>
<td>55.6</td>
<td>8</td>
</tr>
<tr>
<td>Muscle</td>
<td>29</td>
<td>22</td>
<td>75.9</td>
<td>22</td>
</tr>
<tr>
<td>Ex vivo Thyroid</td>
<td>77</td>
<td>24</td>
<td>31.2</td>
<td>51</td>
</tr>
<tr>
<td>Ex vivo Parathyroid</td>
<td>25</td>
<td>14</td>
<td>56.0</td>
<td>29</td>
</tr>
<tr>
<td>Other</td>
<td>7</td>
<td>2</td>
<td>28.6</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>358</td>
<td>140</td>
<td>39.1</td>
<td>245</td>
</tr>
</tbody>
</table>
The 419 readings available for analysis were stratified by patient and tissue type. If there were multiple readings from one tissue type, the average (mean) of the readings was used. This produced 231 spectra for analysis as detailed in Table 3.4.

### Table 3.4 Final number of spectra available for analysis by tissue type

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Number of spectra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid</td>
<td>53</td>
</tr>
<tr>
<td>Normal Parathyroid</td>
<td>43</td>
</tr>
<tr>
<td>Abnormal Parathyroid</td>
<td>20</td>
</tr>
<tr>
<td>Superficial Fat</td>
<td>10</td>
</tr>
<tr>
<td>Visceral Fat</td>
<td>19</td>
</tr>
<tr>
<td>Muscle</td>
<td>17</td>
</tr>
<tr>
<td>Ex vivo Thyroid</td>
<td>36</td>
</tr>
<tr>
<td>Ex vivo Parathyroid</td>
<td>17</td>
</tr>
</tbody>
</table>

The complete set of raw data is available in the appendix (Appendix 3.7) of this thesis and the final data set used for analysis is shown in Appendix 3.8.

For analysis, both normal and abnormal thyroid data were grouped together as the study was not designed to differentiate between thyroid pathology and the spectra from these tissues were sufficiently similar to be grouped in this way for analysis. The data in Appendix 3.8 is demonstrated in Figure 3.10 (larger versions can be found in Appendix 3.9) and the median spectrum of each tissue is demonstrated in Figures 3.11 and 3.12.

To address the objectives of the study (as detailed in section 3.2), data from parathyroid glands were compared with the other tissues in the neck, normal parathyroid glands were compared to abnormal parathyroid glands and data from *in vivo* and *ex vivo* tissues were compared.
3.4.3 Normalising data

When all the spectra taken from the same tissue were superimposed on the same graph for comparison (Figure 3.9) the spectra showed lower impedances and greater variability in amplitude than that reported in the rabbit model (Antakia, Brown et al. 2016). Possible causes for this may include the presence of blood around the tissues; difficulty placing the probe flat on the tissue of interest; and/or the volume of tissue interrogated by the probe was much less in the rabbits compared to humans.

In order to remove this variability to directly compare the shape of the spectra, the data was normalised to the mean amplitude of each spectrum over all frequencies. This did not affect the changes of impedance with frequency but allowed easier comparison of the different tissues.

As it is the shape of the spectra we are interested in, rather than the absolute values, we required a way to be able to compare them directly. Rather than comparing the raw numbers directly e.g., at frequency x, patient a had an impedance of y and patient b had an impedance of z, a measure of how far away from the mid-point of the spectrum that point is (Figure 3.9).

To normalise a spectrum, the mean across the 14 frequencies was computed (Spectrum Mean, SM) and then each of the 14 values (V) were divided by the spectrum mean giving 14 normalised values (NV). This process is explained with examples in Appendix 3.10 and the normalised data is presented in Appendix 3.11. The normalised spectra from each patient separated by tissue type are demonstrated in Figure 3.13 (detailed versions in Appendix 3.12) and the median normalised spectrum of each tissue is represented in Figure 3.14.
Figure 3.9 Normalising data

In graph 1, two spectra are displayed from the same tissue. Their absolute values of impedance are very different at the same frequency (point a and point b). The spectra are normalised by measuring the distance at each frequency from the mid-point (mean) of the curve to give a value. When these values are compared in graph 2, the shape of the graph is shown to be very similar and point a and b at the same frequency are now shown to be closer together. The shape of the spectra is more easily compared once they have been normalised. Once the spectra have been normalised a new “template” spectrum is made by taking the median value at each frequency from the normalised values.
Figure 3.10  Impedance spectra of individual patients from each tissue.

Where more than one spectrum was available for a tissue in an individual, the mean of the readings at each frequency has been used so only one spectrum is represented for each individual.
Figure 3.1 Median spectrum of the tissues of the central neck.

These spectra represent the median at each frequency of all the available data of each tissue type.
Figure 3.12  Median impedance spectra of thyroid, normal parathyroid and abnormal parathyroid glands with the range displayed at each frequency.

*Individual tissues are displayed below for comparison.*
Figure 3.13  Normalised impedance spectra of individual patients from each tissue. Where more than one reading per individual was available, the mean of the readings was taken and displayed.
Figure 3.14 Median normalised spectra of impedance
3.4.4 Impedance ratios

Antakia et al. found a significant difference in the ratio of low (152 Hz) to high (312 kHz) frequency between the tissues (Figure 3.2) (Antakia, Brown et al. 2016). This corresponded to their observations that in vivo the thyroid appeared to have a higher EI than parathyroid glands at low frequencies (Figure 3.1).

In the current study, the data from the thyroid and parathyroid glands had a much greater overlap than was found in the rabbit study. When the median impedance of human tissues (Figure 3.11) was compared to that from the rabbit model (Figure 3.1), the EI from thyroid and parathyroid tissue in humans were similar at the lowest and highest frequencies whereas there was a greater difference between these two tissues at low frequencies in the rabbit model.

As in the rabbit study, the low to high ratios were compared across the tissue types. The results are demonstrated as a box and whisker plot in Figure 3.15. There is a statistically significant difference in the low to high ratio of thyroid, normal parathyroid and muscle using Friedman’s 2-way analysis of variance by ranks test (p = 0.006).

Data on impedance of the tissue in the central portion of the spectrum was not analysed by this ratio. The impedance of the tissues at low and high frequencies were similar but the pattern of impedance changes over the frequencies appeared different as demonstrated by the shape of the spectra. The impedance of the thyroid started to decrease at a lower frequency when compared to the parathyroid producing an inverse sigmoid shaped spectrum. The parathyroid tissues maintained a higher impedance over a broader range of the lower frequencies and demonstrated a steep decline in impedance at the higher frequencies. Comparing the spectra from the rabbit and human studies, it appeared that the shapes were fairly similar, but the range of amplitude was narrower in humans. Developing a
method of comparing the shape of the curve may help differentiate parathyroid tissue from the other tissues in the neck.

### 3.4.5 Template matching

In the development of the probe for use within colposcopy procedures, cervical templates were made by 3D cellular modelling based on the histopathology and morphology of the specific tissue giving the “ideal” impedance of that tissue. The spectra taken from research subjects in the cervical studies were then compared to the artificial template to see how closely they matched (unpublished data) (Tidy, Brown et al. 2013, Murdoch, Brown et al. 2014). A similar artificial template was not

![Box and whisker plot comparing the ratio of in situ electrical impedance at two frequencies (152 Hz and 312 kHz) in thyroid, normal parathyroid and muscle in humans (p=0.006). The ends of the box represent the 25th and 75th quartiles; the line in the box represents the median; the whiskers represent the minimum and maximum values.](image)

**Figure 3.15** Comparison of in situ ratios of human thyroid and parathyroid glands

*Box and whisker plot comparing the ratio of in situ electrical impedance at two frequencies (152 Hz and 312 kHz) in thyroid, normal parathyroid and muscle in humans (p=0.006). The ends of the box represent the 25th and 75th quartiles; the line in the box represents the median; the whiskers represent the minimum and maximum values.*
produced for this study given the complex nature of thyroid and parathyroid tissue structure and the logistical limitations of this small study. To provide a template for this study, the median normalised spectra for each tissue group was used to form the template (Figure 3.14). Comparing the mean and median spectra of each tissue type showed a close relationship and confirmed the researcher’s opinion that the spectra have a normal distribution around the mean with few outliers.

Each of the normalised spectra was then analysed by template matching to the appropriate tissue template. The template match is the deviation from the template expressed as a fraction. For each normalised value (NV) across a spectrum the template match (TM) was calculated using the template value (TV) (Appendix 3.11). This provided the template match figure across 14 frequencies. The mean taken across the spectrum, formulated the spectrum template match (STM) and was expressed as a number between 0 and 1. The closer the value is to 0, the closer the spectrum matched the template. The degree to which the spectrum for a tissue matched to a template was compared by converting it to a relative probability. A threshold was set and the number of spectra matching, i.e., less than or equal to the threshold, at that value, was determined. By repeating this process over many thresholds, a graph of template matching was produced that demonstrated how closely each tissue matched the template.
Figure 3.16  Graph of template matching of each spectrum across 14 frequencies. This compares the normalised spectrum of a tissue to the median normalised spectrum of the normal parathyroid tissue to compare the tissues.

A) Thyroid to normal parathyroid template

B) Normal parathyroid to normal parathyroid template
3.4.6 Receiver Operating Characteristic (ROC) Curve

Template matching across thresholds can be used to calculate the sensitivity and specificity of template matching and produce a ROC curve (Brown, Tidy et al. 2000). This is helpful to determine the threshold for the template match. To ensure the effectiveness of this approach, a decision should be made about the key objective of the test. For instance, when comparing normal parathyroids and thyroid to the Normal Parathyroid (NP) template, increasing sensitivity will increase the number of correctly identified parathyroids but also increase the number of false positives (thyroid). Increasing the specificity, to reduce the number of false positives, will decrease sensitivity and fewer parathyroids will be correctly identified. Therefore, a balance between sensitivity and specificity must be sought. ROC curves plot sensitivity against 1-specificity and assist identifying the balance (Figure 3.17). Using our data, thyroid and normal parathyroid tissue were distinguished with a sensitivity of 76% and specificity of 60%.

Figure 3.17  ROC curve for normal parathyroid vs thyroid using the correlation with the normal parathyroid template
3.5 Discussion

3.5.1 Findings of this study

The difference in EI spectra between the thyroid and parathyroid was not well-defined in humans as in the rabbit model. Overall, the readings from human tissue showed significantly lower impedance than those taken from the rabbit tissue. The reason for this is unclear but may be related to the presence of circulating blood in the region of interest. In the rabbit model, at low frequencies there was a large difference in impedance with the thyroid having a much higher impedance than the parathyroid gland. In the human study, this was not the case. There was a much greater overlap in impedance and the thyroid and parathyroid spectra were unable to be separated on the impedance values alone. There was also a greater variability of the amplitude of impedance within the tissues compared to that taken in the rabbits. This variability was greater at low frequencies and improved as the frequency increased. For example, the impedance of normal thyroid at 76 Hz has a range of 530.30 Ω (176.83-707.13 Ω), whereas at 625 kHz the range is 94.61 Ω (72.89-167.50 Ω). The same is seen in the normal parathyroid where the range at 76 Hz is 433.68 Ω (138.99-572.67 Ω) and at 625 kHz is 207.61 Ω (44.19-251.80 Ω). This is likely to be multifactorial but the presence of blood around the probe and tissues could be a factor. Surgeons use water to clear the operative field of blood stain for better visualisation of the parathyroids. Water has a very high impedance so if the tissues were surrounded by residual water this may have affected the impedance. The volume of tissue being interrogated by the device is larger in humans and may have been influenced by disease.

However, there are similarities between the findings in the human and rabbit study, in relation to the shape of spectra. Normalising the spectra of the human data to the mean amplitude of each spectra reduces the dependency of the surrounding fluid and tissue volume. Normalising the data removed the variability in the amplitude but maintained the changes in impedance with frequency. As seen in the rabbit model, the human parathyroid spectra exhibited a much flatter curve than the thyroid, maintained the same impedance over the low frequencies which then reduced
rapidly at higher frequencies. The thyroid spectra, in comparison, had a more inverse sigmoid shape with a rapid decline in the mid-range frequencies. The impedance at the lowest and highest frequencies of human parathyroid and thyroid tissues were similar but the pattern of reduction in impedance with increasing frequency was not different. The data available from both superficial (subcutaneous) and deep (visceral) fatty tissue, however, showed a similar shaped curve to that of the parathyroid.

Template matching gives a good indication of how well the shape of a specific curve matches the template. Template matching across 14 frequencies demonstrated areas of the spectrum where the greatest differences and similarities to the template are. Template matching is scored between 0 (complete match) and 1. The normal parathyroid spectra matched well to the template (Figure 3.16B), especially at frequencies <78 kHz where template matching for all spectra is <0.09, mean 0.016 (range 0-0.086). This was also seen when comparing both superficial and visceral fat to the parathyroid template. Comparison of the abnormal parathyroid spectra to the normal parathyroid template showed good template matching at the lower frequencies and was <0.06 at frequencies up to 39 kHz (mean 0.019, range $0.7 \times 10^{-4}$-0.060). Comparison of the thyroid spectra to the normal parathyroid template is where the greatest differentiation in template matching was seen (Figure 3.16A). The degree of template matching was lower than that seen in both parathyroids and fat samples except at the mid-range frequencies where all spectra had a high degree of template matching (Mean template match thyroid to NP template over all frequencies is 0.065 with a range 0-0.372 and at 4.8-19.5 kHz 0.027, range 0-0.21). This corresponded with the area of the thyroid spectra where the impedance starts to have a rapid decline and crosses the parathyroid spectra which is continuing to remain steady.

The difference in the spectra from the template can be exaggerated by weighting frequencies that had the greatest difference in the template match. When comparing the thyroid and normal parathyroid to the NP template the frequencies that demonstrate the greatest difference in template matching are 76, 152, 305, 610, 1220, 19531, 39062 and 78125 Hz. The amount of weighting applied had been arbitrarily
chosen as a 2, 4 and 8. This would change the total number of spectra matching at each threshold. The template matching of the unweighted and weighted data is compared against the templates (Figure 3.18). The weighted template matching of the normal parathyroid spectra to the NP template was very similar to that of the unweighted data, however, there has now been a visible change in the template matching of the thyroid to the NP template.

![Graph](image)

**Figure 3.18  Percentage template match**

*Percentage template match of thyroid and normal parathyroid to the normal parathyroid template over increasing thresholds and the effect weighting certain areas of the spectrum.*

This was a simplistic method of weighting the data and other complex methods which may be more statistically acceptable are available. Similar work using EIS in other applications is being conducted within the University of Sheffield and complex statistical modelling to analyse the shape of the spectra collected is forming a part of their analysis. These models have not yet been tried on our dataset, but discussion
with expert analysts suggest that the characteristic shape of the spectra is the most important feature for analysis and may help differentiate between tissues more.

In agreement with the findings in the rabbit study, *ex vivo* spectra had a higher impedance than the *in vivo* readings of the same tissues (Figure 3.11), most likely due to the change in temperature and motility of ions. Mean *in vivo* temperature was 36.3°C and *ex vivo* temperature was 27.2°C. This increase in impedance was seen in both the parathyroid and thyroid tissues. The increased impedance was greatest at lower frequencies and the impedance of the tissues were similar to *in vivo* results at the highest frequencies. The overall shape of the spectra was maintained, and this is best seen in the normalised data where the *ex vivo* parathyroid data is closely approximated to the abnormal parathyroid data. The majority of the *ex vivo* parathyroid glands will have been those excised due to disease so it is expected that it would fit more closely with the *in vivo* abnormal glands than the normal glands. The *ex vivo* thyroid spectra are also closely associated with the *in vivo* thyroid spectra. Differences between *in vivo* and *ex vivo* spectra may help in the detection of devascularised parathyroid glands. Although the median spectra show an increased impedance at low frequencies, there is a large range of impedance and only 4 of the 17 of the *ex vivo* parathyroid spectra had a higher starting impedance than the *in vivo* spectra. 15 parathyroid data sets were available where *in vivo* spectra can be directly compared to *ex vivo* spectra due to exclusions. 71% of *ex vivo* spectra had a higher impedance at the lowest frequency compared to *in vivo* spectra from the same patient.

At surgery, it is often difficult to determine with confidence that a gland is devascularised and requires auto-transplantation but a high impedance or an increase in impedance in the same gland after a period of time (which may be variable but in the region of minutes to hours) may indicate devascularisation. The *in vivo* temperature was taken using a temperature probe placed in the oesophagus. This measures the central temperature. It is likely that the actual temperature of the exposed tissues in the neck are lower than the central temperature. The *ex vivo* temperatures were taken from the specimen when it had been removed entirely from the body. The effect of devascularisation on temperature of tissue that remains
in the body cavity is not clear. It is likely that the ex vivo specimen will have a lower temperature than a devascularised in vivo tissue as there will be heat transfer from surrounding tissues and it remains under the operating lights which also transfer heat. Whether there is enough of a temperature difference between in vivo vascularised and devascularised tissues to produce a significant change in impedance to inform the user whether the gland requires auto-transplantation remains to be seen and warrants further investigation. However, we know that the overall shape of the curve does not change between in vivo and ex vivo tissues so devascularised glands will still be able to be identified from the surrounding tissues.

3.5.2 Technical

The ZedScan™ is designed for use in colposcopy and its design is ergonomically appropriate for this application. Its long nose cone allows the cervical tissue to be assessed whilst the screen is easily visible to the operator and the lights on the nose cone are within the eye line of the operator. However, these characteristics were a hindrance in its use in neck surgery. As the tissues of interest are fairly superficial, the screen was difficult to visualise when the tip of the probe was in contact with the tissues. This problem was exacerbated by light from overhead operating lights reflected from the sterile plastic sheath. The buttons on the probe are relatively flat making them easy to clean. However, they were difficult to use in surgery as they were out of view of the user and palpation via the sterile sheath and gloves was difficult. The surgeon was often reliant on the audible feedback beep to know if the button had been pressed. This is difficult to hear in a relatively noisy theatre environment. There is no facility to increase the volume of beep. The other signal of a reading being taken was the lights on the nose cone, but these were often difficult to see due to the brightness of theatre lights and their position on the nose cone. These problems can be appreciated in Figure 3.5 where the surgeon is taking a reading: his hand is not appropriately positioned to take the reading; the screen and nose cone lights are facing away from him; and there is a lot of glare from overhead lights on the cover.
The diameter of the tip is 5.5 mm and we found in most cases the tissues of interest were large enough for a reading to be taken. Normal sized parathyroids and lymph nodes are usually at least about the same size as the probe tip (Figure 3.19) and other tissues including the thyroid have a larger surface area to take a reading. There was however one case where the parathyroid gland was too small to allow complete coverage of the tip of the probe.

The other concern is the reliability of readings taken from parathyroid glands located superficial to other tissues and the potential for these other tissues to influence the EI reading especially when the tip may not be fully in contact with parathyroid tissue alone.

The sterile cover adds to the bulk of the tip. Without the cover, the probe has four delicate gold prongs. These come in contact with the ceramic plate at the end of the cover which makes the tip smooth. Reducing the size of the tip would allow for more accurate placement of the tip on the region of interest.

The sterile cover tended to occasionally become dislodged from the tip. This initiates a warning sign on the screen and stops the surgeon from taking a reading. This resulted in the need to steady the cover in addition to holding the tip at the desired
pressure on the region of interest. Prior to future studies, the cover requires modification to keep it more secure and prevent dislodgement during surgery.

On occasion, the probe lost patient identifiable data after placing in the sterile cover before any readings were taken. This required the handset to be handed back out and re-docked to re-enter this data before proceeding. This is in addition to the two occasions where EIS data had been collected and subsequently lost from the handpiece before it was docked on to the computer.

The current single layer of packaging which opens with a tear across the top is susceptible to break in sterility. This was monitored closely during the study to ensure that sterility was preserved. A good alternative would be a peel pouch such as those that small, lightweight loose surgical instruments are packaged in.
There was a single adverse event when the ceramic tile at the end of the probe cover came loose and separated from the plastic cover (Figure 3.20). This was noticed immediately, and a prophylactic dose of intravenous antibiotics given in line with the trust protocol for contravening asepsis. The incident was documented in the patient notes and study site file and was explained to the patient the following day. No adverse events were noted.

Figure 3.20  Ceramic tile at the end of the sterile cover became dislodged during use

Changes to the design of the instrument in line with the above observations would improve user compliance and ease and potentially improve the quality of data collected. These changes could potentially include the use of a monitor/screen outside the surgical field (to be easily visualised by the operating team), a reduction in probe size and improving the battery life enabling a long surgical time. The handpiece could be packaged separately and sterilised so the cable (connecting the handpiece to the monitor) is handed out and connected to the machine leaving the
tip still sterile for the surgeon to use. The tip of the probe would then not require two layers, could potentially be less bulky and have fewer parts that could not come apart. The tip could be modified to reduce the area of contact and to reduce potential trauma from the electrode tips. With these changes, the handpiece would also require fewer buttons as they would only need one to take the reading and perhaps a second to move through secondary functions such as confirming screen information or to move onto another reading. The way the data is collected and analysed can also be changed to better suit use in neck surgery. At present all 12 readings are taken and then the analysis is done and areas for biopsy highlighted. In neck surgery it would be more useful to have an interpretation done after each reading. For instance, following placement on a tissue of interest, the probe should be able to take a reading and interpret it in real time. This may not need to be stored. However, storage can be facilitated if the handpiece was connected to the laptop and monitor. Data could also be entered by staff in theatre directly on the computer without breaking the sterile field. This had been discussed with the company, and with discussion of the results of this study, there are no current plans to alter the design specifically for use in neck surgery.

3.5.3 Impact of reinstating QC checks

Following reinstatement of the second layer of quality check (as explained in the methods), there was a significant decrease in the number of recordable spectra from both superficial and visceral fat. Prior to quality checks, 30 spectra were recorded on ‘superficial fat’ from 30 patients and 20 of these were rejected on analysis (66.7%). After reinstatement of QC, only 2 spectra were recordable on ‘superficial fat’ from 24 patients and 1 of these were rejected on analysis (50%). All 24 patients had a reading attempted on 3 occasions before moving on to a different tissue. This is thought to be due to fat having a very high impedance and was also seen in the rabbit model (Antakia, Brown et al. 2016). This could have an implication on taking readings from parathyroid glands that have a covering of fat. With QC checks in place, it took multiple attempts before a reading was accepted by the probe on parathyroid glands in 2 patients. The spectra were then later rejected on analysis for one patient. In a further three patients, readings could not be taken on
parathyroid glands despite multiple attempts. All of these parathyroid glands were documented by the surgeon as having a normal appearance but with a covering of fat. This is concerning as a parathyroid could be mistaken for a lobule of fat. In addition, attempts at increasing gland exposure may increase the amount of handling of the gland and increase the risk of devascularising the gland.

3.5.4 Limitations

Unlike in the rabbit model, the tissue sample from which the readings were taken could not always be confirmed on histology as normal tissue would not be removed as part of the patient’s planned procedure. The final diagnosis of ‘normal’ tissue is therefore reliant on surgical judgement. In abnormal glands however, all glands that were positively identified as a parathyroid were confirmed on histology. One gland that was suspected to be ‘parathyroid’ was shown on histology to be thymic tissue.

This study is a phase 1 trial investigating the feasibility of using this technology in human thyroid and/or parathyroid surgery and so the numbers are limited and were not decided a priori. The rejection of unsuitable spectra, particularly from some tissues such as fat, furthered reduced numbers available for analyses.

One of the neck structures we planned to include in this study were lymph nodes as these are structures that can look very similar to parathyroid glands. In the course of this study, we were only able to take readings from two lymph nodes that the surgeons had confidently identified. This was not sufficient for analysis and comment.

We have used our own data to provide the template used in the template matching. There will be an inherent increased matching of the parathyroid spectra to the median of all the parathyroid spectra but if there was a great variability in the shape of the spectra obtained from parathyroid glands, the degree of template matching would still be low. It would be ideal to engineer tissue which was representative of parathyroid tissue to use to create the templates, but this was not feasible in this early trial. This could be considered if this technology is to be developed and retested in a larger study.
3.6 Conclusion

There are similarities in EI spectra of thyroid and parathyroid tissues in humans and rabbits. The primary finding is that thyroid and parathyroid glands can be differentiated using electrical impedance spectroscopy. In the rabbit model the thyroid and parathyroid could be separated using the difference in raw impedance alone as the impedance at low frequencies was much higher in the thyroid than the parathyroid but were similar at high frequencies. In the human model this was not apparent. There was far greater overlap of raw impedance and greater variance in the amplitude of impedance. Therefore, an absolute value of impedance cannot be used to define the tissue type. There are multiple factors within the human study which may account for this greater variability such as blood in the field, gland size or pathology and technical issues whilst using the device. Further analysis has demonstrated that the shape of the curve over the increasing frequencies is likely to help in tissue differentiation. Changes to the design of the device along with complex mathematical modelling of the data is likely to improve the differentiation.

Ex vivo readings did demonstrate a significant increase in impedance compared to in vivo readings probably related to devascularisation and/or reduction in temperature. However, the shape of the spectra was similar; allowing the gland to be identified correctly. This alteration in impedance may be useful in identifying ischaemic glands that may benefit from auto-transplantation, but this needs further investigation.

This technology has the potential to provide a fast and simple answer for a surgeon to confirm the presence of parathyroid tissue. This could be used as an adjunct to the surgeon’s expertise or alongside other identification techniques such as near infrared florescence imaging. This would be of use in both thyroidectomy and neck explorations for hyperparathyroidism where parathyroid glands need to be identified either for preservation or resection. This also has potential to reduce risk of hypoparathyroidism in other head and neck surgeries such as laryngectomy or pharyngectomy.
Chapter 4: Assessment of quality of life in patients with long term Post-Surgical Hypoparathyroidism (PoSH) – A cross sectional observational study
4.1 Introduction

4.1.1 Background to the study

Some complications or consequences of surgery are easier for patients to understand such as bleeding or voice change. However, the impact of disease, its treatment and some complications on the patient’s everyday life is harder to communicate. To attempt to measure outcomes beyond morbidity and mortality, health-related quality of life tools have been developed (Karimi and Brazier 2016). These measures were very basic in design when first utilised in the early 1970s. One of the first tools designed was the Health Status Index (Fanshel and Bush 1970). It provided a generic measure of health and was one of the first tools to judge a patient’s state of health on societal values rather than economics alone. This scale was crude and patient states were defined in a range from “well-being” to “disabled” to “death” (Fanshel and Bush 1970).

A definition of health was provided by the World Health Organisation (WHO) (WHO 2014). They described it as “a state of complete physical, mental and social well-being, and not merely the absence of disease and infirmity”. This definition had great influence on the development of health outcome measures such as the SF-36, which included social well-being as a measure of health (Ware and Gandek 1998). Whilst morbidity outcomes are easy to determine and are relatively binary, quality of life is on a continuum and is subjective. It is affected by individual expectations, human needs and personality. In a paper published by WHO, quality of life was described as “an individuals’ perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns” (Willem 1995). As quality of life is inherently subjective, attempts to include an aspect of objectivity into measures by including social achievements.

The SF-36 Health Survey is a multi-purpose, short form health survey comprised of 36 questions (Ware and Gandek 1998). These questions can be divided into eight domains or health concepts taken from the forty health concepts investigated in the Medical Outcomes Study (MOS) (Stewart and Ware 1992). These eight domains have
been shown to be affected by disease and treatment and are the most commonly included parameters in health surveys (Ware and Gandek 1998). They include physical functioning, physical role limitations, bodily pain, general health perceptions, energy/vitality, social functioning, emotional role limitations and mental health. The SF-36 includes both objective and subjective ratings of health and functioning. Objective measures include specific measures, for example when measuring physical activity patients are asked if they can kneel, climb a flight of stairs, lift groceries, or walk a mile. Patients are also asked more subjective questions such as their level of tiredness. The eight domains can be broadly divided into either physical or mental health groups.

There are many health concepts that are not addressed in the SF-36 including sleep adequacy, sexual functioning, eating, communication or family functioning (Ware and Gandek 1998), therefore, depending on the impact on quality of life one particular health measure could ignore an important outcome to patients. The importance of individual domains may also change dependent on the nature of the intervention being studied. For patients undergoing knee replacement, the physical functioning, bodily pain and physical role limitations are likely to be of most interest to the clinician. However, in the treatment of depression, mental health, social functioning and emotional role limitations may be more notable (Ware and Gandek 1998).

Over recent decades this importance of quality of life as a research outcome has increased and is now recognised as a principal endpoint in health research, particularly in developed countries (Haraldstad, Wahl et al. 2019). Understanding the impact on quality of life of certain treatments or interventions is important in medical decision-making. There is a greater understanding that in some cases, treatment does not always improve quality of life. A systematic review by Haralstad et al in 2019 showed that the majority of quality of life studies focused on cancer or other long-term diseases (Haraldstad, Wahl et al. 2019). Improved medical treatment has led to more patients living with chronic disease and therefore the impact of this on patients’ quality of life is of interest to clinicians. Most quality of life studies were in adults with only 12% including children or adolescents (Haraldstad, Wahl et al. 2019). This is thought to be due to the challenges in assessing quality of life in this age
This review also found that most studies used a generic measure alone or in combination with a condition-specific measure. The papers where a combination was used appeared to be more clinically relevant (Haraldstad, Wahl et al. 2019). Generic measures included the widely used and validated SF-36, EQ-5D and WHOQOL-BREF for adults and Kidscreen, CHQ and PedsQL for children.

A systematic review published in 2017 by Büttner et al reviewed five papers assessing quality of life in hypoparathyroid patients (Büttner, Musholt et al. 2017). This review found that patients with hypoparathyroidism (HPT) receiving standard treatment had an impaired quality of life (QOL) compared to both the general population and other matched patient controls (Arlt, Fremerey et al. 2002, Sikjaer, Moser et al. 2016). It appears that despite being on stable treatment with calcium and vitamin D supplements, patients still experience a significantly reduced quality of life when compared to other patients with long term medical conditions such as Addison’s disease or congenital adrenal hyperplasia (Arlt, Fremerey et al. 2002, Løvås, Loge et al. 2002, Nermoen, Husebye et al. 2010) and report a quality of life similar to those with chronic heart disease or diabetes (Büttner, Musholt et al. 2017). PTH receptors have been identified in the brain, central nervous system and muscle (Balabanov, Töllner et al. 1984, Divieti, Inomata et al. 2001, Bagó, Dimitrov et al. 2009). It is hypothesised that this (rather than hypocalcaemia) is the cause for lower reported quality of life within the domains of mental health, anxiety and fatigue. PTH receptors in the brain have been linked to modulation of the stress response and potentially has an effect on the release of pituitary hormones such as growth hormone as vasopressin (Dobolyi, Dimitrov et al. 2012). The studies included in this review used generic quality of life measures that are not specific to hypoparathyroidism. These questionnaires may have overlooked some significant issues important to patients suffering with HPT. Furthermore, many patients included in the studies had hypoparathyroidism not attributed to surgery. Only two of the five studies included in the review compared patients with PoSH to matched cohorts of surgical patients without PoSH (Arlt, Fremerey et al. 2002, Sikjaer, Moser et al. 2016). The other studies evaluated patients with hypoparathyroidism against national reference data. Although it may be important to compare patients with whole populations, using control groups who have had similar underlying diseases is probably more
appropriate as this helps in attributing reduction in quality of life to PoSH. The two papers (Arlt, Fremerey et al. 2002, Sikjaer, Moser et al. 2016) where PoSH patients were compared to surgical patients without PoSH only had small numbers of participants (n=50 and n=44 respectively). Thus, caution must be exercised when drawing conclusion from these studies alone. The review recommended studies evaluating the consequences on QOL in patients with HPT with disease specific questions such as aetiology, co-morbidity and other related factors. To the best of our knowledge, no in-depth study of quality of life in post-surgical hypoparathyroidism has been conducted in the UK.

4.2 Aims

The aim of the study was to perform a detailed assessment of symptoms and quality of life in different cohorts of patients with post-surgical hypoparathyroidism and to compare these patients with similar cohorts of patients without hypoparathyroidism. This study will take into account the aetiology of hypoparathyroidism, treatment and co-morbidities to analyse for possible predictive factors.

4.3 Methods

4.3.1 Study Design

This was designed as a cross-sectional observational questionnaire study of patients, conducted via an online platform. Participants with post-surgical hypoparathyroidism and appropriate controls (those without PoSH) were identified from four different sources:

1. Butterfly Thyroid Cancer Trust (BTCT) – This is a UK national patient charity whose members have been diagnosed with thyroid cancer. The vast majority of patients have had thyroid surgery. Of these, a small proportion have PoSH as a result of surgery and the remainder served as a control cohort.

2. Association of Multiple Endocrine Neoplasia Disorders (AMEND) – This is a UK national patient charity for patients with MEN disorders. A significant proportion of
patients/members of this charity have had thyroid and/or parathyroid surgery, with some having PoSH. Participants for both the disease and control groups were recruited from this membership.

3. Patients who have undergone surgery for benign or malignant thyroid disease or for primary hyperparathyroidism in the Sheffield endocrine surgery unit (Sheffield Teaching Hospitals NHS Foundation Trust (STHNFT)) from 2010 to 2016 were screened. Patients who have ongoing PoSH were recruited to take part in the study. A random selection of age, sex and ‘year of surgery’ matched patients without PoSH who had thyroid and/or parathyroid surgery were identified from the operating theatre database to serve as controls.

4. Patients with thyroid cancer, MEN1 or MEN2 syndrome attending the follow up thyroid cancer clinics at Weston Park hospital and the endocrine clinics at STH were invited to take part as participants in the ‘disease’ and ‘control’ groups depending on whether they had PoSH or not, respectively.

4.3.2 Ethical approval

The project and the accompanying documents were approved by the East of Scotland Research Ethics Service (EoSRES) Research Ethics Committee (REC Reference: 18/ES/0091, IRAS Project ID: 245001) and the project was registered with and approved by the Research and Development department in Sheffield Teaching Hospitals (STH reference: STH 20208).

4.3.3 Participant Eligibility

Patients from the above four sources (BTCT, AMEND, STH surgical database and MEN / thyroid cancer clinics) were eligible to participate if they had previously undergone thyroid and/or parathyroid surgery. Patients were excluded if they were below the age of 18, were unable to understand spoken and written English or unable to give adequate informed consent.
4.3.4 Participant Recruitment

Patients were identified from the following cohorts and invited to participate:

- **Cohort A** – Charity members of BTCT and AMEND
- **Cohort B** – Patients identified with PoSH and matched controls from the 2010-2016 electronic surgical database
- **Cohort C** – Patients identified from the thyroid cancer clinic who were not already identified in cohort B
- **Cohort D** – Patients identified from the MEN clinic who were not already identified in cohort B

To recruit participants to cohort A, members of the AMEND and BTCT charities were invited to participate in the study via the charities’ websites, newsletters, and social media. A link to the online survey was detailed on the invitation.

Patients for cohort B were identified from electronic operating records in Sheffield Teaching Hospitals. Between January 2010 to December 2016, 1854 endocrine surgical operations were recorded. Patients were included if they had a bilateral neck exploration or total thyroidectomy (n=932) and excluded if they only underwent a hemi-thyroidectomy, unilateral neck exploration or targeted parathyroidectomy (n=922) as these latter groups of patients are not at risk of long-term hypoparathyroidism. Deceased patients were excluded (n=57). The electronic records of the remaining patients (n=875) were accessed to review the pre- and post-operative blood results, discharge medications and readmission data to screen for hypocalcaemia and hypoparathyroidism. Clinic letters of patients discharged home on calcium and/or vitamin D supplements or readmitted with hypocalcaemia to identify patients who needed treatment for more than 6 months after surgery were also reviewed. Sixteen patients were identified as having long-term postsurgical hypoparathyroidism. Each of these patients were matched with three other patients from the same records who were of the similar age (date of birth within 12 months of the index patient), same gender and had a similar procedure in the index year (n=48). It was thought that patients with PoSH may be more inclined to participate in this study than patients without and therefore to ensure a matched
control group more patients were invited in the non-PoSH group. A total of 64 patients were invited to participate in cohort B.

Patients who attended the thyroid cancer clinics were identified from clinic appointment schedules (clinic codes JW22T and HCPJW21A). Between 1st January 2017 and 31st December 2017 910 appointments were scheduled. There were 534 patients after exclusion of duplicates, who were compared with the surgical database and 107 patients already identified in cohort B were excluded. Seven deceased patients were excluded. In total, 420 patients were cross matched with a existing list of patients diagnosed with thyroid cancer from 2000 to 2014 to ensure they had surgery as part of their treatment, which resulted in the identification of 287 eligible patients. Surgical details from patients prior to 2000 are not held electronically and are only available from individual patient’s records, which are often not available. For this reason, only patients who underwent surgery between 2000 and 2014 were included. Many patients having follow up appointments had their operation before the year 2000 but as the extent of their surgery could not be ascertained from the electronic databases available, they were excluded from the study.

Patients attending the MEN clinic were identified from clinic appointment schedules (Clinic code JDN2E) in the manner previously described. Of 119 appointments between 1st January 2017 and 31st December 2017, 100 patients were identified and compared with the surgical database to exclude duplicates. The electronic patient records of 89 patients were then accessed to determine whether they had surgery as part of their treatment and to exclude any deceased patients. Patients attending this clinic may not have had surgery relevant to this study or may have had their operation at another hospital as patients from the surrounding region will have been referred to STH as a tertiary centre. Clinic letters, where available were reviewed. Patients were included in the study if their relevant surgical history was detailed in the letters available. Paper patient records were not requested for full review. 16 patients were identified to be invited to participate in cohort D.

Potential participants from cohorts B, C and D were invited to take part in the survey through an invitation letter posted to their address. The invitation included details of
the study, how to access the survey and the approximate time taken to complete the survey (Appendix 4.3).

These four cohorts of patients were identified as within the groups of patients there will be patients who have had surgery but not had PoSH to act as internal controls. There is likely to be patients within each cohort who have had temporary and long-term hypoparathyroidism.

4.3.5 Online Questionnaire

The online questionnaire was made available through the REDCap platform which could be completed via computer, mobile or tablet devices linked to the internet. REDCap is a secure web application for conducting online questionnaires. On the invitation letters, patients had the option to request a paper copy of the questionnaire, which was then posted out for completion, with a return envelope.

Once the questionnaire was accessed online, participants had to review a detailed information sheet and a consent page before being able to start the questionnaire.

The questionnaire (Appendix 4.4) included demographic details, details on surgery and treatment for hypoparathyroidism, a quality-of-life survey (modified SF-36 quality-of-life measure), questions on symptoms of hypoparathyroidism (including the hypocalcaemia symptom score - HcSS) and other questions relating to patients’ perceptions on the impact of the diagnosis.

The online questionnaire only enabled access to relevant questions, relevance being determined by answers to previous questions. For example, if the patient indicated that they had thyroid surgery then further detailed questions were asked about the surgery; and questions about parathyroid surgery would remain hidden. Some responses, such as consent, were mandatory, while others, such as demographic information, were optional.

The questionnaire was divided into five sections (Appendix 4.4). The first section included optional demographic details such as name, gender, date of birth and contact information for them or their GP if further information was needed. The
second section was about their surgery and included details of the operation, nature of gland(s) removed, number of operations, years since surgery, indications for surgery and pathology. Participants who had undergone thyroid surgery were also asked about other treatments for the primary condition (such as radioiodine or radiotherapy), nature and duration of treatment for hypocalcaemia and for a list of their current medications. The third and fourth sections included questions relating to the SF-36 and HcSS scales respectively. The SF-36 is a 36-question short form health survey developed by RAND Healthcare, which is a verified, generic and easily administered set of quality of life measures (Ware and Sherbourne 1992). The HcSS is a list of symptoms directly linked with hypocalcaemia complied by endocrine surgeons and key members of the Parathyroid UK charity. The final section of the questionnaire asked the participant if they had been diagnosed with any health conditions linked to hypoparathyroidism since their operation.

The questionnaire was available online for 8 weeks for participants to complete and a reminder letter was sent out before the end of the 8-week period.

Prior to the study, representative members of the Parathyroid UK, AMEND and BTCT charities boards were asked to comment on the research design and documents including the invitation, information sheet, consent form and the questionnaire, for suitability, clarity and accessibility. They made recommendations for changes to language and display to increase the comprehension and ease of use. Any comments received were used to revise the documents accordingly. The REDCap website was trialled by members to ensure it could be navigated without problem and that the time taken to complete it was realistic. As the SF-36 is already validated, no further assessment of validity was undertaken. The HcSS was designed to be completed in the same way as the SF-36.

4.3.6 Data analysis

Patients were divided into PoSH and non-PoSH groups based on the prevailing BAETS definition of long term hypoparathyroidism – requirement for calcium and/or vitamin D supplements at six months after surgery (Chadwick, Kinsman et al. 2017). This assessment was made by the investigator (SH) based on the patient’s responses and
current medications. If a patient was currently on alfacalcidol or calcitriol (active vitamin D) and were at least 6 months after surgery, they were considered to have PoSH. If patients were prescribed D3, D2 (inactive vitamin D) or calcium supplements then this alone was insufficient to ensure the diagnosis of PoSH as these medications are used to treat other conditions such as osteoporosis.

To analyse the SF-36 scores, the responses of all participants were recoded giving each question a maximum mark of 100 for good health and 0 for bad health. The SF-36 was divided into 8 domains including physical functioning (PF), role limitations due to physical health (RP), role limitations due to emotional problems (RE), energy/fatigue (vitality, VT), emotional well-being (mental health, MH), social functioning (SF), bodily pain (BP) and general health (GH).

As not all patients answered every question on the SF-36, the denominator was adjusted, and a percentage score was determined. The HcSS score was converted in an identical manner, so each question was marked from 100 to 0 (asymptomatic – symptomatic). The 12 HcSS questions can be categorised into two groups. The first six questions concerned symptoms associated with hypoparathyroidism and the latter six questions related to diagnoses associated with hypoparathyroidism or the treatment of hypoparathyroidism.

Statistical analysis was performed using SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Clinical characteristics of participants were conveyed by descriptive statistics including age, gender, pathological reason for surgery. Differences in the SF-36 and HcSS scores between PoSH and non-PoSH groups were analysed using the Mann-Whitney U test for two group comparison and the Kruskal-Wallis’ test to compare the three groups of patients – thyroid cancer, MEN disease and non-MEN benign pathology. Spearman’s rho was used to analyse any correlation between outcomes from the SF-36 subdomains and HcSS scores. Differences were considered statistically significant at p<0.05.
4.4 Results

In total, 584 responses were logged on the REDCap website. The responses were categorised as being from five ‘sources’ (Charities n= 440, MEN clinic n= 6, Thyroid cancer clinic n= 122, Surgical database PoSH n= 4, Surgical database controls n= 12). Of these, 145 responses were excluded for various reasons - duplicate responses (n= 13), SF-36 not completed (n= 104) and consent not given (n= 22). If the SF-36 was incomplete and no demographic information was provided, the response was excluded as duplicate response could not be ruled out (n= 5) (Figure 4.1). Duplicates were identified through names, emails and date of birth provided by the participants. If two responses from the same participant were identified then the response with the higher completion percentage or, if identical, the first response was chosen. For the final analysis, 439 responses were included from the five sources (Charites n= 322, MEN n= 6, Thyroid Cancer n= 95, Surgical PoSH n= 4, Surgical control n= 12). These were then divided into four groups dependent on their pathology - thyroid cancer (n=252), MEN (n= 60), non-MEN benign disease (n= 120) and those where the pathology was unclear (n= 7). Within these groups, the patients were divided into PoSH and non-PoSH (Table 4.1) as described in the methods (see section 4.2.5). The participants where the pathology was unknown were excluded from further analyses.

Responders were predominantly female (female n=379, male n=52, undisclosed n=8) with a median age of 52 at the time of response (range 19 - 92). The reported operation dates ranged from 1973 - 2019. 347 out of 439 responses had a fully complete SF-36.
Figure 4.1  STROBE diagram for recruitment and allocation to pathological groups

Figure legend: Cohort A: Charites (BTCT and AMEND), Cohort B: Surgical database
Cohort C: thyroid cancer clinic Cohort D: MEN clinic
Table 4.1  Number of responses to the survey stratified by pathology, gland(s) operated and PoSH vs non-PoSH

<table>
<thead>
<tr>
<th>Gland(s) operated</th>
<th>Groups</th>
<th>Thyroid Cancer</th>
<th>MEN</th>
<th>Non-MEN &amp; Benign</th>
<th>Unsure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid</td>
<td>PoSH</td>
<td>31</td>
<td>5</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Non-PoSH</td>
<td>190</td>
<td>6</td>
<td>64</td>
<td>0</td>
</tr>
<tr>
<td>Parathyroid</td>
<td>PoSH</td>
<td>0</td>
<td>11</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Non-PoSH</td>
<td>0</td>
<td>20</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>Both</td>
<td>PoSH</td>
<td>10</td>
<td>11</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Non-PoSH</td>
<td>19</td>
<td>7</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Unsure</td>
<td>PoSH</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Non-PoSH</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

The median (interquartile range) reported quality of life in the overall dataset (n=439) in PoSH and non-PoSH groups were 54.2% (37.2 - 76.8%) and 60.6% (39.9 - 79.1%) respectively (Mann Whitney U test; p=0.348). The comparisons between the groups within the various subdomains are shown in Figures 4.2 and 4.3. There was no significant difference in any of the domains, with the exception of the vitality (energy/fatigue) subdomain where the scores were significantly lower in the PoSH group (p=0.008). Further subgroup analyses between PoSH and non-PoSH patients were performed for the individual pathological groups and these are shown in Figure 4.2. Again, there was no statistical significance in the overall quality of life (p=0.540). Scores in the energy/fatigue domain were significantly lower in the thyroid cancer (p=0.032) and MEN (p=0.029) groups, but not in the ‘other benign’ group (p=0.620).

The SF-36 scores in the three groups (Thyroid cancer, MEN and non-MEN benign) were compared using the Kruskal-Wallis test. This showed statistically significant differences between groups in overall quality of life and all subdomains except for the ‘role limitations due to emotional problems’ domain (p=0.146) (Table 4.2). The non-MEN benign pathology group reported the lowest quality of life in all eight domains. The responses of the eight domains and overall SF-36 score ranged from
40-85 in the thyroid cancer group, 25-95 in the MEN group and 25-65 in the benign group. The highest scoring domain across all three groups was the physical functioning domain. The worst scoring domains were role limitations due to physical health and vitality.

Questions on the HcSS score were answered by 419 participants. Participants with PoSH (n= 88) reported significantly more symptoms (p<0.001) associated with hypoparathyroidism than those without (n= 331). As shown in Figure 4.2, the HcSS scores were significantly lower in all participants with PoSH compared to those without (p<0.001), and also in the thyroid cancer (p=0.004), MEN (p= 0.006) and non-MEN benign (p=0.019) groups. The responses to individual HcSS questions were then compared between the PoSH and non-PoSH groups of all respondents and between the three pathological groups.
Figure 4.2  Comparison of the overall SF-36 score, its domains and the HcSS in patients with and without PoSH in the overall cohort and groups stratified by pathology.

Figure legend: median, 25th and 75th percentile of percentage SF-36 score. SF-36 = overall SF-36 score, PF = physical functioning, RP = role limitations due to physical health, RE = role limitations due to emotional problems, VT = energy/fatigue (vitality), MH = emotional well-being (mental health), SF= social functioning, BP = bodily pain, GH = general health, HcSS = overall score
Figure 4.3  Comparison of the overall SF-36 score and the VT (energy/fatigue) domain in patients with and without PoSH in the overall cohort and groups stratified by pathology.

Scores range from 100 (asymptomatic/good health) to 0 (symptomatic/poor health)
Figure 4.4  Comparison of the overall HcSS score and its components in patients with and without PoSH in the overall cohort and groups stratified by pathology.

Scores range from 100 (asymptomatic/good health) to 0 (symptomatic/poor health).
Table 4.2  Comparison of the overall SF-36 score and its domains in patients across groups stratified by pathology.

<table>
<thead>
<tr>
<th></th>
<th>Thyroid Cancer n=252</th>
<th>MEN Syndrome n=60</th>
<th>Benign Pathology n=120</th>
<th>Significance (Kruskal-Wallis’ test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF-36</td>
<td>62.6 (44.4 - 79.9)</td>
<td>61.2 (33.8 - 83.5)</td>
<td>48.5 (31.5 - 68.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PF</td>
<td>85 (60 - 100)</td>
<td>90 (44.3 - 100)</td>
<td>65 (31.3 - 90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RP</td>
<td>50 (0 - 100)</td>
<td>25 (0 - 100)</td>
<td>25 (0 - 100)</td>
<td>0.023</td>
</tr>
<tr>
<td>RE</td>
<td>66.7 (0 - 100)</td>
<td>83.3 (0 - 100)</td>
<td>33.3 (0 - 100)</td>
<td>0.154</td>
</tr>
<tr>
<td>VT</td>
<td>40 (25 - 60)</td>
<td>40 (18.8 - 60)</td>
<td>30 (10 - 50)</td>
<td>0.003</td>
</tr>
<tr>
<td>MH</td>
<td>65 (48 - 80)</td>
<td>66 (44 - 81)</td>
<td>60 (40 - 72)</td>
<td>0.010</td>
</tr>
<tr>
<td>SF</td>
<td>75 (50 - 100)</td>
<td>62.5 (50 - 88)</td>
<td>62.5 (38 - 75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BP</td>
<td>67.5 (45 - 100)</td>
<td>67.5 (35 - 90)</td>
<td>55 (34.5 - 78)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GH</td>
<td>50 (25 - 70)</td>
<td>40 (20 - 65)</td>
<td>35 (20 - 53.8)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Figure legend: median (25th and 75th percentile) of percentage SF-36 score. SF-36 = overall SF-36 score. PF = physical functioning. RP = role limitations due to physical health. RE = role limitations due to emotional problems. VT = energy/fatigue (vitality). MH = emotional well-being (mental health). SF= social functioning. BP = bodily pain, GH = general health
The median SF-36 and HcSS outcomes were compared by decade in patients where the year of operation was provided (Table 4.3). There was no statistically significant difference in either the SF-36 or HcSS scores across the decades for patients with PoSH. The SF-36 and HcSS scores varied within the non-PoSH group with patients having had operations in the years 2000 to 2009 showing the highest outcomes for quality of life and least symptoms of hypoparathyroidism and those having had operations in the years 2010 to 2019 reporting the lowest quality of life and highest symptoms in the HcSS. These differences were found to be statistically significant using the Kruskal-Wallis test (SF-36 p=0.005, HcSS p=0.002).

Participants were asked about their blood test results within the last 6 months. 88 out of the 89 participants with PoSH had their calcium monitored. There was a variety of responses regarding the control of calcium levels but only 43% reported that their calcium was within the normal range. 44% had ‘low’ or ‘very low’ calcium and 3% reported a ‘high’ calcium. Most patients with PoSH (n= 82, 92%) also had a PTH test in the prior 6 months, although fewer patients (59%) were able to recall the result. Of those who recalled the result, 39% reported PTH to be ‘low’ or ‘very low’ but no patients reported a ‘high’ PTH. Only 12% of non-PoSH (n=39) stated their calcium was ‘low’ or ‘very low’ and 20 patients stated their PTH was ‘low’. Nine of these patients with reportedly low PTH in the non-PoSH group were on some form of treatment: five were prescribed calcium and inactive vitamin D, three inactive vitamin D and one patient had calcium supplementation alone. Data from the STHNFT patients was validated. Of the 117 STHNFT patients, 80 had provided consent and their survey responses had enough information to review their medications and blood test results. All 11 (100%) patients who self-identified to have PoSH were prescribed alfacalcidol. Of 7 patients with PoSH who reported their calcium to be ‘low’ or ‘normal’, five patients were correct. One patient reported ‘normal’ calcium and had a low calcium and PTH, one reported ‘low’ calcium but had a low PTH with normal calcium.

Eight patients allocated to the non-PoSH group had stated they were on treatment for hypocalcaemia but were not on active vitamin D based on the medication list they
provided. After validation of their medication from their GP, one of these patients was prescribed alfacalcidol, which had not been included in their self-reported medication list. All other patients were on low dose vitamin D supplements or calcium for bone health.

Of the patients who consented and had their data validated, accuracy of self-stratification and allocation to the PoSH group was 100% (11/11) and 98.6% (71/72) for the non-PoSH group.
Table 4.3  Comparison of overall SF-36 and HcSS scores in patients with and without PoSH across decades

<table>
<thead>
<tr>
<th>Decade</th>
<th>SF-36</th>
<th>HcSS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PoSH</td>
<td>Non-PoSH</td>
</tr>
<tr>
<td>&gt;1999</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF-36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PoSH</td>
<td>59.2 (29.2-84.3)</td>
<td>68.4 (41.4-86.1)</td>
</tr>
<tr>
<td>n=10</td>
<td></td>
<td>n=12</td>
</tr>
<tr>
<td>2000-09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF-36</td>
<td>50.8 (39.0-75.1)</td>
<td>73 (47.6-84.7)</td>
</tr>
<tr>
<td>n=27</td>
<td></td>
<td>n=81</td>
</tr>
<tr>
<td>2010-19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF-36</td>
<td>56.2 (35.5-75.9)</td>
<td>56.3 (37.4-75.6)</td>
</tr>
<tr>
<td>n=50</td>
<td></td>
<td>n=244</td>
</tr>
<tr>
<td>Significance (Kruskal-Wallis' test)</td>
<td>0.935</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Figure legend: median (25th and 75th percentile) of percentage SF-36 and HcSS score
Figure 4.5  Correlation of HcSS and VT domain

*Graph depicting moderate correlation between hypocalcaemia symptom score (HcSS) and energy/fatigue domain of the SF-36 in 88 patients with PoSH (Spearman’s rho correlation co-efficient 0.497, p<0.001)*
4.5 Discussion

Patients with PoSH clearly have an impaired quality of life as shown by several recent original articles (Arlt, Fremerey et al. 2002, Cusano, Rubin et al. 2013, Sikjaer, Rolighed et al. 2014, Astor, Løvås et al. 2016, Sikjaer, Moser et al. 2016, Wilde, Wilken et al. 2020) and a systematic review (Büttner, Musholt et al. 2017). The key studies are summarised in Table 4.4. The majority of the studies have used validated quality of life questionnaires that are not specific to hypoparathyroidism. These include the Short Form 36 (SF-36), WHO-5 Wellbeing Index survey (WHO-5) and the Hospital Anxiety and Depression Scale (HADS). These studies found that patients with PoSH have significantly reduced quality of life when compared to the general population. However, the direct comparison of quality of life in PoSH patients to the general population may not be valid as PoSH occurs in the context of underlying thyroid/parathyroid disease; this disease and the consequences of surgical treatment will have an impact on quality of life.

Matched control studies only include very small numbers of patients; the study by Arlt et al compared 25 females with PoSH following goitre surgery with 25 females without PoSH following the same surgery (Arlt, Fremerey et al. 2002). Sikjaer et al compared 22 PoSH patients post thyroidectomy with 22 post thyroidectomy and 22 healthy individuals without PoSH (Sikjaer, Moser et al. 2016). The other studies with a larger sample size do not use matched controls but use normative data as a control. Normative data acts to provide a baseline distribution for a score or measurement based on large, randomly selected samples. It is not, therefore, representative of the population under investigation.

Studies comparing SF-36 in patients with PoSH to normative data show that patients with PoSH report a significantly decreased quality of life in at least 7 out of 8 domains (Cusano, Rubin et al. 2013, Sikjaer, Rolighed et al. 2014, Astor, Løvås et al. 2016). In the study by Sikjaer et al post thyroidectomy patients both with and without hypoparathyroidism were compared to healthy individuals. Where the two thyroidectomy groups are compared, fewer differences were reported in quality of life (Arlt, Fremerey et al. 2002, Sikjaer, Moser et al. 2016). In Sikjaer et al, when
patients with PoSH were compared to healthy individuals, quality of life was significantly lower in 7 out of 8 domains of the SF-36. There was no significant difference in the role limitations due to emotional health (RE) domain. However, the same study showed that when post thyroidectomy patients with and without PoSH were compared there is only a significant (p<0.05) difference in 2 out of 8 domains, physical functioning (PF) and role limitations due to physical health (RP).

The recent published studies either do not include or have very small numbers of patients with PoSH following parathyroid surgery. In addition, the definition of PoSH is not clearly described/defined in these papers. As concluded in the systematic review (Büttner, Musholt et al. 2017) and in the recent study where a new tool, the Hypoparathyroid Patient Questionnaire (HPQ) has undergone validation testing (Wilde, Wilken et al. 2020), studies using generic quality of life tests, such as the SF-36, in patient and controls, may not have assessed PoSH-specific symptoms.

Four main factors are thought to influence quality of life; being in a follow up programme, the effect of the disease on daily activities, coming to terms with the diagnosis and uncertainty concerning the future (Strømsvik, Nordin et al. 2007). Health related quality of life has previously been investigated in patients who have undergone thyroidectomy for thyroid cancer. Studies have shown that patients report a lower quality of life than matched controls (age, gender and socioeconomic status) from the general population (Lee, Kim et al. 2010, Li, Zhang et al. 2020); the reduced quality of life has been linked to anxiety regarding recurrence despite this disease having a good prognosis and low incidence of recurrence. However, the number of co-morbidities also significantly decreases the reported quality of life (Hedman, Djärv et al. 2016), as in the general population (Djärv, Wikman et al. 2013). Patients with MEN disease have a reported a lower quality of life than the general population (Berglund, Lidén et al. 2003, Correa, Farias et al. 2019).
<table>
<thead>
<tr>
<th>Reference</th>
<th>Population</th>
<th>Definition of PoSH</th>
<th>Control</th>
<th>QoL measures</th>
<th>Key findings</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arlt, Fremerey et al. 2002 (Arlt, Fremerey et al. 2002)</td>
<td>Women with PoSH after goitre surgery or parathyroidectomy for PHPT (n=25); PoSH on stable treatment</td>
<td>Treatment with calcium and active vitamin D supplements for &gt;6 months following surgery</td>
<td>Women without PoSH after thyroid surgery (n=25); matched for age and time since surgery</td>
<td>ACL-90-R GBB-24 B-L Zerssen</td>
<td>Higher global complaint score (GBB-24 p=0.036, B-L Zerssen p=0.002, SCL-90-R p= 0.020) Predominant increase in anxiety</td>
<td>Small study numbers, parathyroid disease excluded</td>
</tr>
<tr>
<td>Astor, Løvås et al. 2016 (Astor, Løvås et al. 2016)</td>
<td>PoSH documented by ICD-10 codes (n=197, F=161) – preceding surgery undefined</td>
<td>Treatment (undefined) for &gt;1 year following surgery where serum calcium below reference range with simultaneously low or inappropriately normal PTH</td>
<td>Norwegian national normative data</td>
<td>SF-36 HADS</td>
<td>Significantly lower SF-36 scores and higher symptom scores for anxiety and depression in PoSH patients.</td>
<td>Unmatched controls Surgical intervention is not defined</td>
</tr>
<tr>
<td>Sikjaer, Rolighed et al. 2014 (Sikjaer, Rolighed et al. 2014)</td>
<td>Total (n=62 F=53), PoSH (n=56) – atoxic goitre (n=25), toxic goitre (n=17) Cancer (n=12), PHPT (n=4)</td>
<td>Treatment with active vitamin D or high dose D2 for &gt;1 year where serum calcium below reference range with simultaneously low or inappropriately normal PTH</td>
<td>US National normative data</td>
<td>SF-36 WHO-5</td>
<td>PoSH patients had significantly lower scores in all domains (p&lt;0.05). No significant difference in WHO-5 scores.</td>
<td>Surgical intervention not defined. No matched controls.</td>
</tr>
<tr>
<td>Sikjaer, Moser et al. 2016</td>
<td>PoSH post thyroidectomy (n=22, F=3)</td>
<td>Treatment with active vitamin D or high dose D2 for &gt;1 year where serum calcium below</td>
<td>Post thyroidectomy without PoSH (n=22), healthy</td>
<td>SF-36 WHO-5</td>
<td>PoSH had lower scores in all SF-36 domains except role-emotional subdomain.</td>
<td>Small sample size.</td>
</tr>
<tr>
<td>Study</td>
<td>Design Description</td>
<td>Outcome Measures</td>
<td>Results/Interpretation</td>
<td></td>
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<tr>
<td>(Sikjaer, Moser et al. 2016)</td>
<td>reference range with simultaneously low or inappropriately normal PTH</td>
<td>Individuals matched for gender and age at time of testing (±2 years) and time of surgery (±2 years) (n=22)</td>
<td>WHO-5 showed no difference between the two surgical groups (p=0.72), but both had a lower score than the controls (p&lt;0.01)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Cusano, Rubin et al. 2013</td>
<td>Total (n=54, F=40), PoSH (n=27) Surgical intervention undefined</td>
<td>Treatment with calcium and vitamin D for &gt;6 months for serum calcium and PTH concentrations below the lower limits of normal on at least 2 occasions separated by at least 30 days Chronic HPT defined as &gt;2 years.</td>
<td>SF-36</td>
<td>Significantly lower scores in all subdomains (P&lt;0.001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cusano, Rubin et al. 2013</td>
<td></td>
<td>US National Normative data</td>
<td>Small sample size, no matched controls Surgical intervention undefined</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Wilde, Wilken et al. 2020</td>
<td>PoSH following thyroid surgery (n=49, F=36) (Total thyroidectomy/subtotal/near total/hemi and subtotal)</td>
<td>presence of hypocalcaemia and inappropriately low PTH levels, and patients needing treatment (undefined) at least 6 months after surgery 39 following thyroid surgery (as for population and hemithyroidectomy) 35 hyperparathyroidism matched for gender and age</td>
<td>HPQ 40/28</td>
<td>Identification of specific symptoms in patients with HPT differed to controls. Unvalidated tool Extent of surgery not matched i.e., total thyroidectomy and hemithyroidectomy. Underlying pathology not matched e.g., carcinoma, Graves’ disease</td>
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</table>
The current study evaluated 439 patients following surgical treatment which could predispose to post-surgical hypoparathyroidism and compared quality of life in those with and without PoSH. Quality of life may be influenced by several factors including the underlying pathology which required the surgery, co-existing morbidity, ongoing management and other treatment related morbidity. For this reason, cohorts chosen from different sources were categorised into groups on the basis of underlying pathology – thyroid cancer, MEN syndromes and others.

The results show that hypocalcaemia related symptoms are (as expected) worse in patients with PoSH compared to those without. We have also shown that patients with PoSH report a significantly lower level of energy and increased fatigue. As shown in Figure 4.4, there is a moderate correlation between the HcSS questions 1-6 (symptoms of hypocalcaemia) and the energy/fatigue domain of the SF-36.

Results from this study differ from those previously described in that the difference in quality of life is reduced to one domain. Power calculations were not done prior to commencement but this study includes more participants than all the studies described previously combined. Therefore it is unlikely that the study is underpowered and can account for this result.

Development of renal calculi and renal failure was significantly higher (p=0.041 and p<0.001 respectively) in patients with PoSH compared to those without. These are conditions closely associated with long-term hypoparathyroidism (Underbjerg, Sikjaer et al. 2013). Other diagnoses (such as seizures, renal failure and cataracts) have been described as occurring more frequently in patients with PoSH, compared to the general population (Shoback 2008, Underbjerg, Sikjaer et al. 2013, Vadiveloo, Donnan et al. 2019). In this study, the reported incidence of these diagnoses did not differ between the PoSH and non-PoSH groups. This may be another area where comparing groups that are otherwise similar (except the diagnosis of PoSH) reveals the true impact of PoSH.

Treatment of patients with PoSH is primarily aimed to address symptoms. Patients with PoSH included in this study were all on treatment (as this was required to include them in the PoSH group). The cause for persistence of symptoms despite
treatment is not fully understood and could be due to either inadequate or ineffective treatment, low compliance, the direct effects of low PTH or a combination of these issues. Guidelines on the management of long term PoSH emphasise the importance of maintaining calcium in the low normal range (Bollerslev, Rejnmark et al. 2015) and this may partly account for ongoing symptoms in some patients. It is possible that increasing the dose of calcium and/or vitamin D may improve symptoms in these individuals, but this may increase the risk of hypercalcaemia, hypercalciuria and its associated long-term morbidity.

The majority of patients with PoSH had regular monitoring of their calcium and PTH levels with blood tests and showed their calcium to be within the normal or low ranges. However, ongoing medical treatment, and even good calcium control, does not fully address hypocalcaemia related symptoms as these patients did report an increased number of hypocalcaemia symptoms compared to those without PoSH. This indicates potential for improvement in current management strategies.

Despite patients with PoSH having significantly more symptoms compared with the non-PoSH group and a moderate correlation between the degree of symptoms on HcSS and energy/fatigue scores (Figure 4.2), this has not translated into a significant reduction in overall quality of life. This may either be due to lack of a large enough sample size (type II error) or due to the impact of other comorbidity in these patients. However, there was a consistent and statistically significant reduction in scores in the energy and fatigue domain as reported by patients with PoSH. It is possible that patients who were experiencing severe symptoms of hypoparathyroidism and therefore having a negative effect on quality of life would seek support from a charity such as Parathyroid UK. If members from this charity were included in the study, there may have been a more significant impact on quality of life.

The surgeon’s awareness of the importance of parathyroid function and its preservation during thyroid surgery has increased over time (Dralle 2015). This is evidenced by changes in surgical techniques, the use of novel technologies and patient monitoring. However, standard surgical techniques have only changed
marginally in recent times. Routine parathyroid auto-transplantation, proactive identification of parathyroid glands at surgery and routine subtotal thyroidectomy have all been tried in an attempt to reduce post-surgical hypoparathyroidism (Delbridge 2002, Delbridge 2003). The current recommendation is that the glands should be identified wherever possible and preserved. If the vascular supply is compromised, auto-transplantation into a well vascularised site such as muscle is recommended (Perros, Boelaert et al. 2014). In patients with parathyroid disease, pre-operative scanning and identification of single gland disease has improved, resulting in less invasive, unilateral or targeted operations. This reduces the risk of post-surgical hypoparathyroidism.

A number of novel technologies have been researched and developed over the past 10 years to augment the ability of macroscopic, naked eye identification of parathyroid glands. Technologies such as methylene blue or indocyanine green fluorescence aim to aid identification and prevent damage to the glands with the potential to improve patient outcomes and post-operative quality of life. The use of formal protocols for the early detection, management and monitoring of patients with PoSH both in the short term (Wang, Roman et al. 2012) and in the long term (Bollerslev, Rejnmark et al. 2015) also indicate an improved understanding of the incidence and potential morbidity of this condition. Awareness of vitamin D deficiency, correction of pre-operative vitamin D levels and careful post-operative monitoring enables timely initiation of treatment and appropriate follow up, thus patients are treated before the symptoms of hypocalcaemia become severe. Most patients cease treatment soon after or at least within six months of surgery, for example BAETS report that the post-operative hypocalcaemia rate for patients undergoing first time thyroid surgery between 2010-2015 was 10.2%, but only 3.6% of these patients required calcium supplements at six months (Chadwick, Kinsman et al. 2017, Edafe and Balasubramanian 2017). Careful follow up of patients with monitoring of calcium, magnesium, phosphate, vitamin D, PTH and renal function with ongoing adjustments to their medications is important to ensure adequate control of symptoms and limit morbidity of over treatment (Leng, Charlesworth et al. 2014) .
In the current study, SF-36 and HcSS responses from patients with PoSH were analysed by the decade in which their surgery was performed, with no statistical difference in reported QOL (Table 4.3). The median HcSS score, and the SF-36 score for the decades 1990-99, 2000-09, and 2010-19 were similar. However, within the non-PoSH group there was a statistical difference in both the SF-36 and HcSS scores between the decades with the lowest quality of life reported on 2010-19.

4.5.1 Limitations

The questionnaire was designed using one widely accepted scoring system which is deemed reliable (i.e., produces similar results under consistent conditions) and valid (i.e., measures what it intends to). The SF-36 gives a good overall snapshot of a person’s well-being in that moment. Although not used in this study, there are many norms and comparator scores available to act as controls such as the Health Survey for England (Bowling, Bond et al. 1999) and the Oxford Healthy Life Survey (Jenkinson, Layte et al. 1996). The SF-36 takes up to 10 minutes to complete and is intended for self-completion; making it easier to send online and is less time consuming than interviewing participants and noting their responses. However, patient self-completion could lead to some errors by incorrect boxes being marked. The scales within the questionnaire are set up in different orders, ensuring that participants read the responses carefully before making their choice. The data then needs to be modified prior to analysis to generate a score that can be compared between the two systems. This is a process where potential errors can be introduced. The other limitation of this scale is that it has a small range of response options. HcSS (Appendix 4.4) is another simple scoring system that asks about symptoms regularly attributed to hypocalcaemia, some associated with mild symptoms such as tingling in the fingers or around the mouth and some associated with more severe symptoms such as muscle cramps and diarrhoea. There are also conditions which are associated with treatment of hypoparathyroidism such as dry hair or skin. These were explored as part of the HcSS. The HcSS allowed a more detailed understanding of the problems patients are experiencing. As all the responses are restricted to a scale, there is no option for free text to expand or explain the responses. An in-depth interview style questionnaire or focus groups may give more insight into the
reasoning behind the QOL answers but this is a labour-intensive and costly method and one in which many patients may not be willing to participate.

The HcSS is an unvalidated tool and is accepted as a limitation of this study. However, newer tools, discussed in Chapter 6.1, that are undergoing validation studies use similar symptoms as the basis of their scoring systems in the context of hypoparathyroidism.

Parathyroid UK, a UK based charity specifically for those with hypoparathyroidism, collaborated in the design of the study and development of the questionnaire. However, their membership did not participate in the study, as it was not be possible to obtain a valid control group (of patients without hypoparathyroidism) from this cohort.

During the period of data collection, the researchers were contacted by participants to alert us that the website was not working correctly, and they were unable to complete the questionnaire. REDCap was contacted and they were aware of the fault due to a security update and access was re-established. To the best of their knowledge, the website down time was 48 hours. The participants who had emailed directly were thanked for alerting us and advised that it was now live again to participate. Reminder letters were sent out to all potential participants with an explanation that if they had tried to access the questionnaire previously and had experienced problems, these were now rectified.

Data on surgery, medications and other clinical details that were provided by the patient could not be corroborated and this may have introduced errors in interpretation, especially when grouping patients into categories of PoSH and non-PoSH groups. This categorisation depended on patients accurately reporting their medication history. If ‘vitamin D supplements’ were mentioned, this was presumed to be the inactive form and mandated grouping into the non-PoSH group. Consequently, some patients categorised into the non-PoSH groups may have PoSH, but the converse is considered very unlikely.
Participants recruited from the Sheffield surgical database and clinics had their pathological diagnosis and PoSH diagnosis confirmed. In this cohort, the outcomes from the PoSH and non-PoSH groups (overall, thyroid cancer, MEN and benign) were compared. There was no statistical difference seen between the two groups overall or within the pathological subsets. There was also no statistical difference in the hypocalcaemia symptoms reported by the groups. This may be due to the smaller number of participants included in the analysis. Performing the same analysis on the responses from the charities, again showed no statistically significant difference in overall SF-36 but there was a statistical difference in the energy and fatigue domain. The lack of statistical significance in STH groups may be due to the smaller sample size of this subgroup. Alternatively, it may be that patients with post-operative problems seek support from charities and are a self-selecting population with associated bias and increased prevalence of symptoms compared to all patients who have undergone the same surgery.

There are patients within the non-PoSH group who have reported low calcium on blood tests within the last six months and some have had their PTH levels monitored. A proportion of these patients are also taking medications such as calcium and/or inactive vitamin D. The BAETS definition of long-term post-operative hypocalcaemia is the ongoing requirement for calcium ± vitamin D supplements at six months to prevent hypocalcaemia (Chadwick, Kinsman et al. 2017). Patients may be on calcium or vitamin D supplementation for different medical reasons such as osteoporosis not related to parathyroid function or purchase them over the counter. To increase the specificity of patients included in the PoSH group the inclusion criteria was limited to patients taking calcium with active vitamin D. It is possible that patients with low calcium and/or PTH within the non-PoSH group who are taking supplements may actually have long-term hypocalcaemia and be hypo parathyroid. These numbers are low with only 2.6% of responders in the non-PoSH group identifying a low PTH on recent bloods and taking a form of supplementation. The exact results of patients’ calcium and PTH levels have not been validated as part of this survey and results are therefore susceptible to ‘recall bias’. However, patients within the PoSH group do appear to undergo more regular follow up with blood tests than the non-PoSH group, which is as expected.
Although thyroid and parathyroid surgery are the common operations that can cause hypoparathyroidism, any operation in the central neck such as laryngectomy and pharyngectomy can result in PoSH. A recent systematic review (Edafe, Sandler et al. 2020) reported the rates of transient hypoparathyroidism following laryngectomy with concomitant hemi-thyroidectomy or total thyroidectomy ranged from 5.6 to 57.1% and 0 to 12.8% respectively. Higher transient (62.1 – 100%) and long-term (12.5 – 91.6%) rates were reported in patients who also required oesophagectomy. This group of patients may represent a significant proportion of patients with PoSH in the community and have not been included in this study.

The design of this study meant that there would be a greater number of responses from patients without PoSH than with PoSH and those with thyroid cancer compared to less prevalent pathologies. All the data from respondents was utilised in the analyses to avoid selection bias. However, this does mean an uneven distribution of participant numbers between the study groups.

4.6 Conclusion

Overall, patients with PoSH had more symptoms of hypocalcaemia than those without including paraesthesia, muscle cramps and diarrhoea. No statistically significant difference was found in overall quality of life in the SF-36 between the PoSH and non-PoSH groups. PoSH was however associated with statistically significant lower scores in the energy and fatigue domain and had a moderate correlation with the symptoms of hypocalcaemia.

This study has not shown that PoSH has a significant impact on overall quality of life, as reported in previous studies. This study aimed to compare patients with PoSH to a group of patients matched for underlying diagnosis and treatment which has not previously been undertaken in large numbers. This study has shown that hypoparathyroidism does have a significant impact on one specific aspect of quality of life – i.e., patient’s energy levels and ease of fatigue. Whilst the SF-36 gave an overall view of the patient’s quality of life, the HcSS highlighted the additional symptoms and co-morbidities associated with hypoparathyroidism. This shows the
importance of the development of a disease specific QoL tool to expose the problems particular to HPT.
Chapter 5: Post-operative hypocalcaemia and hypoparathyroidism after parathyroid surgery – incidence, severity and management
5.1 Background

Parathyroid hormone (PTH) is produced by the parathyroid glands and in primary hyperparathyroidism (PHPT), there is autonomous and excessive synthesis and release of PTH. This results in high serum calcium through the direct effects of PTH on the kidney and bone and indirect effects of PTH on the gastrointestinal tract. Raised PTH leads to high bone turnover with an overall depletion in bone density causing osteoporosis and increasing susceptibility to fractures (Rolighed, Rejnmark et al. 2014). Patients with PHPT can also present with renal calculi or chronic kidney disease due to hypercalciuria (Khan, Hanley et al. 2017). Other symptoms associated with PHPT are low mood, irritability, increased thirst, polyuria, aches and pains, acid-peptic symptoms, and constipation. In the long term, there is an increased risk of cardiovascular mortality and cerebrovascular disease (Yu, Leese et al. 2013).

Parathyroid surgery (parathyroidectomy) is the standard and definitive treatment for most patients with hypercalcaemia associated with primary hyperparathyroidism (PHPT) (Rolighed, Rejnmark et al. 2014). Transient and/or long-term hypocalcaemia and hypoparathyroidism (PoSH) is a potential complication of parathyroid surgery. There are two main causes of post-operative hypocalcaemia after surgery for PHPT – ‘hungry bone syndrome’ and hypoparathyroidism. Hungry bone syndrome (HBS) is seen post-operatively in patients with severe primary hyperparathyroidism where there has been high bone turnover (Cartwright and Anastasopoulou 2021). After removal of the enlarged parathyroid glands, PTH levels drop rapidly resulting in an influx of calcium into bone previously been depleted in response to the high PTH (Jain and Reilly 2017). This can cause profound hypocalcaemia and is associated with hypophosphataemia and hypomagnesaemia. PTH should be elevated in HBS in response to hypocalcaemia, although in the first instance could be low or normal due to normal parathyroid glands taking some time to recover after being suppressed by the high PTH levels in PHPT. HBS lasts until skeletal bone is remineralised and bone turnover is normalised. Treatment of pre-operative vitamin D deficiency helps to improve bone mineral density before surgery without causing adverse effects (Rolighed, Rejnmark et al. 2014). Pre-operative normalisation with active vitamin D is thought to reduce the severity of post-operative HBS (Cartwright and
Anastasopoulou 2021). The role of bisphosphonates is not clear, but some studies suggest that bisphosphonates do not increase the risk of HBS and may have a protective effect (Lee, Sheu et al. 2006, Mayilvaganan, Vijaya Sarathi et al. 2017). The treatment of HBS aims to replenish the circulating calcium deficit to within the normal range to support bone remineralisation. This is usually achieved with oral supplements, but in some cases of severe hypocalcaemia, intravenous infusions are required. Hypomagnesaemia is also seen in HBS and will require treatment as hypocalcaemia is difficult to treat with ongoing hypomagnesemia (Witteveen, van Thiel et al. 2013). Calcium absorption is also enhanced by the prescription of active vitamin D metabolites or analogues.

The other cause of post-parathyroidectomy hypocalcaemia is post-surgical hypoparathyroidism. In these instances, serum PTH will be low (Cartwright and Anastasopoulou 2021). In post-surgical hypoparathyroidism, there is either intentional excision of 3.5 glands (in patients with multi-gland disease) or damage to or devascularisation of normal parathyroid glands (in addition to excision of abnormal glands): the latter is a consequence of the exploration of the neck in an attempt to find the enlarged gland(s) or to rule out multi-gland disease. Here, recovery takes longer or can sometimes be long term. Patients have hypocalcaemia and require treatment with calcium and/or vitamin D supplementation and further follow up with blood tests. Long term hypoparathyroidism requires on-going care and is associated with long term morbidity. Low PTH levels results in a reduction in the ability to reabsorb calcium via the renal tubular system, leading to hypercalciuria and the development of renal calculi (Edafe, Mech et al. 2019). The recent 5th National Audit by the British Association of Endocrine and Thyroid Surgeons describes a long-term hypocalcaemia (present at 6 months) rate of 3.5% in patients undergoing a bilateral neck exploration and 1.6% for those undergoing a targeted procedure (Chadwick, Kinsman et al. 2017). These are the rates quoted to patients as part of the informed consent process, but the precise rate of this complication in Sheffield Teaching Hospitals NHS Foundation Trust (STHNFT) is not known.

In patients with parathyroid gland suppression due to high pre-operative circulating PTH levels, the normal glands can become atrophic. PTH has a short half-life of 2-4
minutes (Fraker, Harsono et al. 2009, Leiker, Yen et al. 2013) and therefore levels can
decrease before the normal glands recommence production. One study showed that
parathyroid recovery is observed in the majority of patients by 30 hours and is
usually recovered to normal levels by the time the calcium has reached its lowest
point (Brasier, Wang et al. 1988).

Despite hypocalcaemia being the commonest complication following
parathyroidectomy (Mittendorf, Merlino et al. 2004), there is no clear consensus on
post-operative monitoring for patients with PoSH after parathyroid surgery. The
optimal treatment strategy suggested by national guidelines are not supported by
good quality evidence (Edafe, Mech et al. 2019). Two general approaches to treating
post-thyroidectomy hypoparathyroidism have been suggested – splinting and
stimulating (Stack, Bimston et al. 2015). Normalisation of serum calcium with calcium
and vitamin D supplements and allowing the parathyroid gland time to recover is
called 'splinting'. Maintenance of calcium at the lower end of the normal range to
increase the demand for PTH and to stimulate parathyroid cells is dubbed
'stimulating'. Most cases of hypocalcaemia following parathyroidectomy for PHPT will
have an element of HBS and thereby require calcium +/- vitamin D supplementation
at a higher threshold than that after thyroidectomy. Surgeons may also pragmatically
prescribe supplements with the expectation that hypocalcaemia will develop over the
course of days following parathyroidectomy based on their clinical experience. A
review by Edafe et al found that there was no robust evidence available on the effects
of calcium, vitamin D and recombinant PTH in the management of PoSH (Edafe, Mech
et al. 2019). Guidelines from the UK, Europe and the US differ in their approach to
managing these patients due to a lack of good quality evidence on the best treatment
strategies (Edafe, Mech et al. 2019). This has led to variable management across UK
centres (Htun, Edmiston et al. 2018).

Currently, calcium and parathyroid hormone (PTH) levels are now routinely checked
on the first post-operative day following surgery at Sheffield Teaching Hospitals NHS
Foundation Trust (STHNFT). This is to determine if surgery has been successful and
to identify patients with (or at high risk of) post-surgical hypoparathyroidism (PoSH).
This will enable doctors to decide on supplementation with calcium and/or vitamin D
medications. STHNFT guidelines currently in place determine the need for supplementation dependent on the levels of calcium and PTH on the first post-operative day. These guidelines were introduced in 2012 and were revised in 2017 to provide more explicit guidance on commencing supplementation. There have been however instances of patients presenting with clinical and/or biochemical evidence of hypo- or hypercalcaemia during the follow up period. This management protocol (presented as a flow chart in Appendix 5.1) also does not take into account the nuances of surgical findings and likelihood of HBS based on pre-operative clinical features and is likely to over-treat some asymptomatic patients who may recover without intervention and under-treat some patients with normal day one bloods who may manifest with features of HBS after the first post-operative day.

5.2 Aims

To evaluate the risk of transient and long-term post-operative hypocalcaemia and/or hypoparathyroidism rates following parathyroid surgery and to validate (or amend) the existing guidelines on the management of transient post-surgical hypoparathyroidism.

5.2.1 Objectives

To evaluate transient and long-term hypocalcaemia/hypoparathyroidism rates against nationally reported figures.

To assess adherence to existing local guidelines and identify variation and potential reasons for variation from the guidelines.

To identify any areas where improvements could be made to reduce the rate of post-operative hypocalcaemia (by evaluating risk factors and surgical strategies).
5.3 Methods

Patients who underwent parathyroid surgery for PHPT (bilateral neck exploration, unilateral neck exploration and targeted parathyroidectomy) were identified from the endocrine surgery database between 1st November 2012 to 31st August 2017. Blood test results were taken from the electronic computer database as follows; pre-operative adjusted calcium levels, pre-operative vitamin D levels, adjusted calcium and PTH levels on the first post-operative day, adjusted calcium levels at outpatient clinic visit and beyond 6 months after surgery. The number of glands thought to be excised were derived from the operation note and the number of glands identified within the specimens determined from the histopathology report. Details of medications at discharge and follow up plans were derived from discharge summaries and scanned electronic notes. Details regarding readmission to hospital within the 30-day post-operative period and attendances to hospital for post-operative monitoring were also sought from the electronic records. Documentation of ongoing treatment were retrieved from outpatient clinic letters.

This project was approved and overseen by the local Clinical Effectiveness Unit (project number 8898). Data collected was stored anonymously electronically on trust approved, secure, password protected devices. Data was maintained on a Microsoft Excel database and analysis was primarily descriptive.
5.4 Results

Four hundred patients underwent (first time and re-do) surgery for primary hyperparathyroidism between November 2012 and August 2017. Bilateral neck exploration was the most common procedure (n=231) whilst unilateral neck exploration or targeted parathyroidectomy was performed in 169 patients (n=87 and 82 respectively). Pre-operative adjusted calcium ranged from 2.12-3.50 mmol/L (median 2.77 mmol/L). The vast majority of patients (n= 373, 93%) had a pre-operative vitamin D level available. Of these, 28 patients had low vitamin D levels (<20 nmol/L, eight of which were severely deficient (<12.5 ng/mL) on their last pre-operative blood test. This is likely to be an under-estimate of the problem, as many patients would have had treatment for vitamin D deficiency by the time of parathyroid surgery.

The operation notes and histopathology report of all 400 patients comparing the number of parathyroid glands thought to be excised by the surgeon and the number of parathyroid glands identified on histology were concordant in 90% of patients (n=359). There were two patients who had bilateral neck explorations where no abnormal parathyroid glands were seen during surgery. A number of specimens including paraoesophageal nodes and the thymus were excised, but none revealed parathyroid tissue on histology. In the cases where the number of glands excised reported on the operation note and the number of glands identified histologically were non-concordant, there were seven instances where one fewer than the expected number of glands were excised. These were described as lymph nodes (n=2), adipose tissue (n=1) or thyroid tissue (n=4). Twenty-four patients had more parathyroid glands excised than was documented by the surgeon on the operation note. These “additional” parathyroid glands were identified within the specimens in thymic tissue, level 6 tissue or intra-thyroid. Some were inadvertently excised with thyroid tissue or were labelled as a mass of other origin, such as a thyroid nodule. Four patients with inadvertent normal parathyroid excision had also undergone thyroidectomy (hemi-thyroidectomy or total thyroidectomy) as part of their treatment either for thyroid disease or as an en bloc resection of a possible parathyroid cancer. One specimen of a parathyroid gland was histologically
suspected to be two separate glands. The frequency of location of glands identified on histology, but not recognised at the time of surgery, are displayed in Table 5.1. The median number of glands excised in the entire cohort was 1 (range 0 - 4) (Table 5.2).

Three hundred and ninety-five patients were admitted to the ward following the procedure and had at least one overnight inpatient stay. One of these patients was a targeted parathyroidectomy who had been earmarked for same day discharge but stayed in just over 24 hours due to chest symptoms after surgery. He did not have blood tests on the first post-operative day. All (100%) patients who had planned admissions had an adjusted calcium check on the first post-operative day. Overall, 83% of patients had a day 1 PTH check. Since the recommendation to perform PTH was introduced in 2015, the compliance rose significantly from 67% at the end of 2015 to 100% in 2016 and 2017.

Cure rate has been defined as normocalcaemia (2.20-2.60 mmol/L) persisting for six months following surgery (Ishii, Mihai et al. 2018, Ishii, Stechman et al. 2021). Normocalcaemia (2.20-2.60 mmol/L) was achieved in 84% (63/394) of patients on day one blood results (hypocalcaemia n=6, hypercalcaemia n=57). Day one adjusted calcium was ≤2.85 mmol/L in 98% (386/394) of cases. Of the 57 patients who remained hypercalcaemic on day one, 12 continued to be hypercalcaemic on blood tests in the 3-9 month follow up period and was associated with a raised PTH level (>6.9pmol/L). Five patients had an adjusted calcium ≥2.85 mmol/L on their 3-9 month follow up results. Two had been significantly hypercalcaemic (>2.85mmol/L) on day one bloods, the other three patients were previously mildly hypercalcaemic (2.61-2.84mmol/L) on day one results.

Six (1.5%) patients were identified as having an adjusted calcium (adj Ca) of less than 2.1 mmol/L on the first post-operative day (POD1). Based on the recommendation at the time, these patients should have been considered for calcium supplementation. Five patients were discharged on supplements. The patient who was not discharged on supplements had tests repeated the following day and normal calcium levels had been achieved.
Table 5.1  Location of parathyroid glands identified on histopathological report but not identified as parathyroid tissue by the surgeon in 24 patients

<table>
<thead>
<tr>
<th>Specimen as labelled by surgeon</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymic tissue</td>
<td>8</td>
</tr>
<tr>
<td>Thyroid nodule</td>
<td>6</td>
</tr>
<tr>
<td>Intrathyroidal</td>
<td>2</td>
</tr>
<tr>
<td>Level VI tissue</td>
<td>3</td>
</tr>
<tr>
<td>&quot;Mass&quot;</td>
<td>2</td>
</tr>
<tr>
<td>Adherent to thyroid</td>
<td>2</td>
</tr>
<tr>
<td>Single parathyroid</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 5.2  Number of parathyroid glands reported on histology stratified by post-operative biochemistry.

<table>
<thead>
<tr>
<th>Number of parathyroid glands on histopathology report</th>
<th>All cases n = 400</th>
<th>POD1 - low PTH, normal calcium n = 106</th>
<th>POD1 - low PTH and low calcium n = 2</th>
<th>POD1 - normal PTH, low calcium n = 4</th>
<th>POD1 - normal PTH and calcium n = 288</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>272</td>
<td>71</td>
<td>1</td>
<td>2</td>
<td>198</td>
</tr>
<tr>
<td>1.5</td>
<td>1</td>
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<td>2</td>
<td>86</td>
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Note: POD1 – first post-operative day; low PTH - <1.6 pmol/L; low calcium – serum adjusted calcium <2.1 mmol/L.
A total of 58 (14.5%) patients were discharged on calcium (sandocal or Adcal D3) and/or active vitamin D (alfacalcidol). 18.6% of patients who underwent a bilateral neck exploration were discharged on supplementation compared to 11.5% for unilateral neck explorations and 6.1% of targeted parathyroidectomies (Table 5.3). Most patients (93%) who were discharged on supplements had either a low adjusted calcium or low PTH on the first post-operative day. Of the four patients with normal biochemistry on the first post-operative day, three were started on sandocal and one patient was prescribed both sandocal and alfacalcidol. This is likely to be due to the surgeon’s concern that the patient would develop HBS, and the treatment would be considered prophylactic.

**Table 5.3** Calcium and/or vitamin D supplementation at discharge in patients stratified by extent of surgery for primary hyperparathyroidism.

<table>
<thead>
<tr>
<th>Discharge Medication</th>
<th>Bilateral neck exploration n = 231</th>
<th>Unilateral neck exploration n = 87</th>
<th>Targeted Parathyroidectomy n = 82</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandocal/Adcal D3</td>
<td>27</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Sandocal + alfacalcidol</td>
<td>16</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

Seven (1.8%) patients were continuing supplementation six months after surgery; all of these had bilateral neck exploration (7/231 – 3%). No patients who underwent a unilateral neck exploration or targeted parathyroidectomy required treatment beyond six months.

Three hundred and eighty-four of 400 patients had follow up biochemical assessment in this hospital. Four patients were discharged back to their local hospital for follow up. In one patient, adjusted calcium level was not available (haemolysed sample) but the PTH was within the normal range. Information was not available in the remaining 12 patients. Of these, 11 patients, had normal day 1 adjusted Ca and
PTH. One patient had normal adjusted calcium, but a low PTH and their bloods had normalised a few days later. As discussed previously, not all patients were cured following surgery. Twenty-two patients were hypercalcaemic (adjusted calcium >2.6 mmol/L), five of which had significant hypercalcaemia >2.85mmol/L, on their follow up bloods.

All patients with low serum adjusted calcium on the first post-operative day or in the first week after surgery had follow up bloods taken in the three to nine months after surgery. All patients requiring supplementation or low calcium and/or low PTH levels at follow up had biochemical monitoring in this hospital beyond six months.

5.5 Discussion

There is relatively less literature on post-operative hypocalcaemia and hypoparathyroidism after parathyroid surgery compared to PoSH after thyroid surgery and also compared to data on cure and nerve injury rates. A recent systematic review of outcomes following parathyroidectomy for PHPT reported the overall cure rate following targeted minimally invasive parathyroidectomy (MIP) to be 96.9% with a 1.6% recurrence rate (Ishii, Mihai et al. 2018). Singh Ospina et al compared the outcomes of bilateral neck explorations (BNE) and targeted procedures (TP) reported in 88 studies and found the cure rate to be 98% in BNE and 97% in TP (Singh Ospina, Rodriguez-Gutierrez et al. 2016). In this review, recurrent laryngeal nerve (RLN) injury rates were found to be significantly lower in the minimally invasive parathyroidectomy group. RLN injury rates were reported at 0.3% in the MIP group and 0.9% in the BNE group. There was no significant difference in the reported infection and mortality rate between the groups (Singh Ospina, Rodriguez-Gutierrez et al. 2016). Jinih et al also performed a systematic review and meta-analysis comparing outcomes of focused versus bilateral neck explorations and parathyroidectomy for PHPT. The report concluded that the cure and recurrence rates in both groups were similar but that focused explorations were associated with a shortened operation length and fewer overall complications including hypocalcaemia, RLN palsy and haematoma (Jinih, O’Connell et al. 2017). Although hungry bone syndrome and transient relative hypoparathyroidism following parathyroid
suppression are more common than long term PoSH, the latter is still an important outcome following parathyroidectomy, especially after bilateral or re-do procedures (Edafe and Balasubramanian 2017). Despite being an important outcome of surgery, the papers by Ishii et al, Singh Ospina et al and Jinih et al do not include post-surgical hypoparathyroidism in their reviews.

Evidence on commencing supplementation with calcium and/or vitamin D has been largely extrapolated from evidence published on post-thyroidectomy hypocalcaemia (Stack, Bimston et al. 2015). Guidance published from the British Association for Endocrine and Thyroid Surgeons (BAETS) recommends treatment based on adjusted calcium levels taken within 12 hours of surgery and is identical to the recommendation for thyroidectomy patients. BAETS have recommended that each endocrine unit have their own set of guidelines. With the lack of good quality evidence this has led to a variety of practices across the UK (Htun, Edmiston et al. 2018).

There are three main strategies implemented for managing post-operative hypocalcaemia after parathyroid surgery. The first is prophylactic calcium supplementation whereby patients are routinely started on calcium post-operatively, which is subsequently weaned. This prevents the symptoms of hypocalcaemia in the minority who would develop this complication, overtreats the majority of patients and can rarely induce hypercalcaemia. Sabour et al reported results of a retrospective cohort study of 448 thyroidectomy patients which showed that the risk of hypocalcaemia (serum total calcium <7.5mg/dL one day after thyroidectomy) was lower in patients who were empirically treated with calcium and vitamin D (0%) than those treated due to a low (<15pg/ml) PTH (2.3%). However, this study did report that patients with routine treatment have a higher rate of hypercalcaemia (4.5% vs 0%, p=0.006) one day after surgery (Sabour, Manders et al. 2009). Calcium supplementation is relatively cheap but routine use of active vitamin D is more costly (Stack, Bimston et al. 2015). This strategy is beneficial to speeding up discharge from hospital following thyroidectomy and is most widely used to facilitate same-day discharge, a practice not widely adopted in the UK. Empirical supplementation could also be used in same day discharge following targeted parathyroidectomy where
there is a greater chance of post-operative hypocalcaemia given the added risk of HBS.

To treat hypocalcaemia identified after thyroid surgery, there are two schools of thought on levels of supplementation. One aims to adequately replace the calcium and vitamin D and allow a gradual recovery of bruised or ischaemic parathyroid glands. The second aims to stimulate the parathyroid glands by ‘less than adequate’ replacement and to maintain the calcium in the mild hypocalcaemic range. Neither strategy has been convincingly demonstrated to be superior and choice between the two currently appears to be left to the clinician’s discretion (Stack, Bimston et al. 2015). There is very little evidence on the management of hypocalcaemia after parathyroid surgery based on post-operative PTH levels. PTH has a short half-life (2-4 mins (Leiker, Yen et al. 2013)) and therefore will decrease much faster than serum calcium levels that can take up to 72 hours. Although a low PTH can be predictive of later hypocalcaemia in thyroid surgery (Stack, Bimston et al. 2015), there is no consensus on its role in parathyroid surgery (Khan, Waguespack et al. 2011) and its role in parathyroid surgery is extrapolated from literature on thyroid surgery.

From November 2012, a formal guideline was introduced within the Endocrine Unit at STHNFT advising on calcium and vitamin D supplementation based on a specific cut-off of adjusted calcium (adj Ca) on the first post-operative day 1 (POD1). This was based on data from thyroid surgery literature and the clinicians’ experience in the unit. Since then, the guidelines have undergone a minor revision in 2015. In 2015, a POD1 parathyroid hormone level (PTH) was also recommended. These guidelines recommended that patients were started on calcium supplements (sandocal) +/- active Vitamin D (alfacalcidol) based only on adjusted calcium level on POD1, with a threshold of <2.1 mmol/L and not on the PTH. The treatment was not mandated, and treatment decisions were made based on clinician judgement and other factors such as pre-operative biochemistry, operative findings and other clinical parameters. Based on these guidelines, only 1.5% of patients should have been started on supplements, however, 14.5% of patients were actually commenced on treatment. Therefore, it can be deduced that clinicians were using other factors to predict post-operative hypocalcaemia, and not simply the POD1 blood results.
During this audit period, many patients (n=106) had a normal calcium but a low PTH level on the first post-operative day. In the audit period, it was not recommended that these patients were started on calcium or vitamin D supplements. However, 49 patients fulfilling these criteria were discharged on calcium supplements, 17 of these were prescribed active vitamin D concomitantly and two on inactive vitamin D supplements, at the discretion of the surgeon.

Many patients started on calcium and/or vitamin D treatment were brought back for assessment in the 1-2 weeks after discharge. Seven patients, all of whom had low POD1 PTH, had their medication changed or started following this review. Three patients had low normal adjusted calcium but were symptomatic of hypocalcaemia and were also commenced on calcium supplementation. Two patients discharged on treatment had their doses increased, due to presentation with symptoms of hypocalcaemia. One patient discharged without treatment (as the calcium was normal) did require supplements for late occurrence of hypocalcaemia. One patient discharged without supplements was commenced on Adcal D3 five days later despite calcium being over 2.1 mmol/L. The patient had a history of severe osteopenia and therefore the Adcal D3 administered is likely to have been treatment for this, although documentation available is not explicit.

Another patient with a normal calcium but low PTH level was readmitted to hospital due to symptoms of hypocalcaemia and was discharged on calcium and active vitamin D treatment. One patient with normal calcium and low PTH was discharged on Sandocal alone until they returned for planned blood tests on day six and was found to be hypercalcaemic. They were readmitted for intravenous fluids and sandocal was stopped. Their calcium returned to normal and when they were seen in outpatient clinic at eight weeks, their calcium and PTH were within the normal range.

There remains a lack of evidence for the incidence of and management of PoSH following parathyroidectomy as this is a relatively rare complication (Khan, Waguespack et al. 2011). Guidance on commencing post-operative supplementation originates mainly from studies into hypocalcaemia following thyroidectomy. Grodski et al performed a meta-analysis (Group 2007) of four Australian studies of thyroid
surgery which found that an undetectable PTH level four hours after surgery predicted hypocalcaemia with a sensitivity of 48.7%, specificity of 96.7% and positive predictive value of 83.8%. 16% of patients with an undetectable PTH 4 hours after surgery did not develop hypocalcaemia (Group 2007). They concluded that patients with undetectable PTH 4 hours after surgery should be commenced on calcium and vitamin D supplements with close monitoring for hypercalcaemia. The evidence that a low post-operative PTH is a marker for impending hypocalcaemia after thyroid surgery is reflected in the results of this audit on parathyroid surgery.

A recent retrospective study (Liu, Tang et al. 2020) looked at 100 patients who underwent parathyroidectomy for PHPT. The authors concluded that patient age, gender and pre-operative vitamin D were poor predictors of post-operative hypocalcaemia but that the most significant predicting factor was the percentage decrease from pre-operative PTH to the lowest intra-operative PTH after the pathological gland had been removed (Liu, Tang et al. 2020). This is reliant on all patients undergoing parathyroidectomy having intra-operative PTH (IOPTH) samples, which is not routinely performed in the UK. Guidelines published by the UK National Institute for Health and Care Excellence (NICE) in 2019 does not recommend the use of IOPTH in first time surgery, due to cost and lack of proven effectiveness (NICE 2019).

When using IOPTH, blood samples are taken at baseline (pre-incision), 0 minutes and 10 minutes after excision of the suspected gland (Helbrow, Owais et al. 2016). A satisfactory reduction in IOPTH is used to determine complete excision of hyperfunctioning parathyroid material. Adequacy of the reduction may be assessed using various criteria - the Miami criteria mandate a decrease of >50% intra-operative PTH 10 minutes after devascularisation of the parathyroid gland when compared to either the greatest pre-incision or pre-excision serum level (Khan and Lew 2019). The use of IOPTH varies greatly from centre to centre and has a role in both focused and bilateral neck explorations. Some centres have access to the quick IOPTH assay (Jarrige, Nieuwenhuis et al. 2011) allowing rapid processing of samples enabling results to be obtained within minutes. However, in many other centres (as in STHNFT), the samples are taken by hand to the laboratory and processed. The
results take between 30 and 45 minutes to be available. Constraints with the availability of IOPTH and the increased processing time has resulted in the IOPTH assay being used only for targeted/unilateral neck explorations. The rationale for this is that IOPTH will enable detection of multi gland disease which may otherwise go undetected at a limited exploration. At bilateral neck exploration, the exploration of both sides will (theoretically) allow identification and inspection of all four glands. IOPTH will not enable the localisation of ectopic or ‘difficult to identify’ glands that are abnormally enlarged.

The advantages and limitations of IOPTH have been examined by the NICE guidelines published in 2019 (NICE 2019). While confirmation of excision of the pathological gland will reduce failure rates and re-operation for persistent disease; the review committee felt that in first time surgery, IOPTH added little to improving outcomes of surgery. The limitations of IOPTH include added general anaesthetic time and inefficient use of theatre time.

IOPTH still has a role in re-do surgery, and nearby testing would address some of the limitations of lab based IOPTH. If available, IOPTH may also be used for post-operative risk stratification for development of hypocalcaemia and decision making on commencing supplementation and follow up. This may result in over-treatment of patients who may have a low PTH but may never become hypocalcaemic and under treatment of those who had normal results in the first 24 hours but were on a downward trend.

To streamline the management of patients with low PTH on the first post-operative day, it was decided to incorporate this parameter into the Endocrine Unit Guidelines which were subsequently updated in 2017. The 2017 guidance recommends calcium and active vitamin D supplementation for patients with PTH <1.6 pmol/L on the first post-operative day and calcium alone for patients with a normal PTH but low adjusted calcium level (<2.1mmol/L). In patients where the PTH on the first post-operative day is known, 108 patients had a low PTH but only 45% of patients received treatment. Three patients had a normal PTH but low calcium; and two of these were
prescribed supplements. The patient who was not prescribed supplements had their bloods repeated prior to discharge and their calcium had normalised.

This audit has shown good compliance with the guidance in use at the time. However, with the changes instigated in the 2017 guideline, patients with a low PTH but normal adjusted calcium level would also need supplementation at discharge. However, this is likely to have been stopped at the next review in 2-6 weeks’ time. This may have affected the 7 (7/57, 12.3%) patients who required supplementation when they had a ward attender review and may have prevented the readmission (n=1) due to hypocalcaemia. However, this strategy may result in over-treatment. One patient developed hypercalcaemia with treatment (1/49, 2.0%). On balance it appears that treating patients with a low post-operative PTH will prevent symptomatic hypocalcaemia, but these patients must be closely monitored for hypercalcaemia and supplementation should be tapered and ceased as soon as possible.

Most patients had one gland excised (272/400, 68%). There does not appear to be a significant difference (p=0.406) in the number of glands excised between the groups based on post-operative biochemistry (Table 5.2). This is unlikely to be a predictive factor for post-surgical hypoparathyroidism.

The late hypocalcaemia rates are in accordance with the national BAETS audit. In patients undergoing bilateral exploration, late hypocalcaemia was 3% in this unit compared to the national reported rate of 3.5%. In patients undergoing targeted/unilateral parathyroidectomy, late hypocalcaemia did not occur in this series, compared to the national rate of 1.6%.

Given what we understand about the aetiology of long-term hypoparathyroidism, it is surprising that a targeted procedure could cause long-term PoSH. Theoretically, parathyroid glands on the contralateral side should remain undisturbed. Early hypocalcaemia may be explained due to HBS and/or atrophy of normal glands, but this would not normally cause long-term hypocalcaemia. Data entered into the BAETS audit is self-reported and the entries are not validated. It is therefore possible that re-operative surgery has been mis-coded as targeted parathyroidectomy.
Patients undergoing targeted/unilateral surgery are considered to be amenable to day case surgery, where calcium levels on the first post-operative day is not considered mandatory as they will not develop PoSH. They may, however, develop HBS and in which case patients still need to be monitored for symptoms of hypocalcaemia or be prescribed prophylactic calcium supplements. This management is important to consider when implementing a day case surgery protocol.

5.5.1 Limitations

The original aim of the study was to evaluate patients from 2010 to 2016. However, before 2012 there was a considerable amount of missing data as electronic discharge summaries were not yet in use and paper notes had not reliably been scanned into the electronic system. In addition, guidance on PoSH was initiated in 2012. The study period was therefore changed to include patients from November 2012 to August 2017.

There were some instances where paper notes had not been scanned into the electronic system and follow up information could not be obtained. As this was a pragmatic study, management strategies varied between clinicians.
5.6 Conclusion

Post-operative outcomes at STHNFT for parathyroid surgery are similar to, if not below, the national figures presented in the BAETs 5th National Audit. Post-operative hypocalcaemia is multifactorial. Few patients are hypocalcaemic on the day after surgery and reliable predictive factors are needed that guide treatment of patients asymptomatic at discharge.

Low PTH levels on the first day after surgery appears to be a relatively good and easily accessible marker for the development of post-operative hypocalcaemia. All patients who developed hypocalcaemia after an initially normal post-operative adjusted calcium level, had low PTH levels on the first post-operative day. However, this is not specific and many patients with low PTH level on the first post-operative day did not develop symptoms. It is important to monitor patients commenced on supplementation who are normocalcaemic for the occurrence of hypercalcaemia, although this side effect is rare. It is likely that treating patients with a low PTH level on the first post-operative day will reduce the risk of re-admission with hypocalcaemia than increase the risk of hypercalcaemia. Supplementation in this group also addresses negative calcium balance associated with borderline or low bone density secondary to PHPT. This audit validated the addition of post-operative PTH levels in addition to calcium which had been the previous standard practice.

The key to good patient management is a good system for monitoring patients after discharge on an outpatient basis. In the absence of good evidence, local guidelines based on audit data and clinicians’ preferences should be developed. Further studies on the aetiology and management of post parathyroidectomy hypocalcaemia would provide a basis on which national guidance can be developed to coalesce practise.

Emerging data regarding the intra-operative decrease in PTH predicting post-operative hypocalcaemia after parathyroid surgery is interesting and needs to be tested within the UK population.
Chapter 6: Final Discussion and Conclusions
6.1 Quality of life in post-surgical hypoparathyroidism

Long term post-surgical hypoparathyroidism remains a significant consequence of central compartment neck surgery, done for a variety of reasons. Some have cancers, others are predisposed to cancer and have prophylactic surgery, whilst a proportion of patients have benign disease. Although post-surgical hypoparathyroidism is treatable with supplementation, the consequences of the impact on quality of life may be underestimated in the decision making process prior to surgery. In the information leaflet on total thyroidectomy provided to pre-operative patients, potential hypocalcaemia is described as being effectively treated with calcium and/or vitamin D supplements (available at https://publicdocuments.sth.nhs.uk/pil3599.pdf). However, patients who suffer with PoSH, report a significant impact on their quality of life despite effective treatment, which is not described in the available patient information leaflets. A proportion of patients also report an increase in symptoms at both the upper and lower limits of the normal range (ParathyroidUK 2021) and therefore maintaining their calcium levels within the patient’s smaller, ideal range can be difficult without frequent monitoring (Brandi, Bilezikian et al. 2016). Quality of life is multifactorial and often variable between patients (Carr and Higginson 2001). QoL is primarily subjective and is affected by societal and individual beliefs and constructs and influenced by both positive and negative factors (Megari 2013).

Patients who have undergone surgery, have had treatment for cancer or have a predisposition for developing disease, may have a difference in quality of life compared to the general population. Previous studies have not used matched controls, or the studies have small sample sizes (Arlt, Fremerey et al. 2002, Cusano, Rubin et al. 2013, Sikjaer, Rolighed et al. 2014, Astor, Løvås et al. 2016, Sikjaer, Moser et al. 2016).

The quality of life study detailed in this thesis aimed to address the issues identified in previous studies. To date, the current study is the largest, matched control study investigating quality of life in hypoparathyroidism in the literature. This study narrowed the difference in quality of life between the PoSH and non-PoSH groups to
one domain (vitality) within the SF-36 scale and only showed a statistically significant difference in the energy and fatigue area.

In addition to evaluation of quality of life using the generic, validated SF-36 score, the current study used the Hypocalcaemia Symptom Score (HcSS), to ascertain the difference in symptoms experienced by patients with post-surgical hypoparathyroidism compared to surgical patients without this complication. Although the tool is unvalidated, it has demonstrated that patients with PoSH have more symptoms than those without. It is interesting that although the patients in the PoSH subgroup are more symptomatic, this was not reflected in the overall quality of life scores assessed by the SF-36. We have therefore, got to consider that standardised quality of life tools may not be sufficiently sensitive to the effects of hypoparathyroidism. A more disease specific tool, which focuses on the particular areas where quality of life is affected by PoSH, is likely to show a more dramatic and significant difference in quality of life.

To develop a disease specific tool was not within the scope of this thesis. Focus groups or interviews would need to be conducted to analyse the specific symptoms being reported by patients with PoSH. The questionnaire would then have to go through several processes of testing and redesign before a validation study could be performed and then used in a large, control matched, observational study. Since our study was conducted, several studies have attempted to use disease specific tools to investigate the impact of hypoparathyroidism, at least three of which have now been published (Coles, Chen et al. 2019, Brod, Waldman et al. 2020, Wilde, Wilken et al. 2020). Coles et al have developed, compared and assessed the Hypoparathyroidism Symptom Diary (HPT-SD) against several validated tools: the Functional Assessment in Cancer Therapy-Cognitive Function (FACT-Cog) version 3, the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-Fatigue) version 4 and the Hospital Anxiety and Depression Scales (HADS). It was found that there was some evidence of a possible weakness in some of the neuromuscular symptom related questions (Coles, Chen et al. 2019). Almost half of participants (46%) reported their hypoparathyroidism as “severe” or “very severe”, however on individual questions they did not rate their symptoms as serious. The authors commented that this may
be because patients suffer with many mild symptoms and infrequently have severe symptoms or that they take extra supplementation at the outset of the more severe symptoms to curtail them. Brod et al developed the 26-item Hypoparathyroidism Patient Experience Scale-Impact (HPES-Impact) after conducting concept elicitation interviews with clinical experts and patients. The HPES-Impact focuses on four domains – physical functioning, daily life, psychological well-being and social. The HPES-Impact has not yet undergone validation (Brod, Waldman et al. 2020). Wilde et al commented that a significant reduction in quality of life in hypoparathyroid patients has been repeatedly demonstrated when tested using tools such as the SF-36, WHO-5 and HADS and compared to the general population, but matched control studies using these tools have been disappointing. They concluded that a disease-specific tool was required and developed the Hypoparathyroid Patient Questionnaire (HPQ40 and then the shortened HPQ28). Some validation studies have been conducted in German but the English version remains unvalidated (Wilde, Wilken et al. 2020). This study found that patients with hypoparathyroidism reported significantly more pains and cramps, neurovegetative symptoms, numbness, tingling and heart palpitations than the matched control groups. Significantly lower vitality and increased memory problems were also reported.

Quality of life in patients with post-surgical hypoparathyroidism is an area of interest to both clinicians and patients as demonstrated by the number of studies published in recent years. The complexity of the issue is also shown by the number of different tools that have been developed. The recently published studies, along with the results of the quality of life study in this thesis, have consistently shown that there is lower reported quality of life in patients with PoSH when compared to both the general population and matched-controls.

The impact of post-surgical hypoparathyroidism should not be underestimated by clinicians. Even patients who are maintained within the normal calcium range and who have remained steady on supplementation for several years can still experience a low quality of life. These patients may be difficult to manage as their diagnosis has an ongoing effect on all aspects of their life. The numbers of patients with long term post-surgical hypoparathyroidism are low, so many clinicians will not have a large
experience of their management. There is an argument that patients with hypoparathyroidism should be followed up regularly by a clinician who routinely manages PoSH patients. Patients should have a plan in place for what to do when their symptoms become more significant, such as access to blood tests or a temporary increase in their medication. Clinicians should also have an invested interest in weaning patients off their medication if possible, even after a number of years, to reduce the co-morbidity which has been shown to be associated with hypoparathyroidism and its treatment.

6.2 Peri-operative management of hypocalcaemia and hypoparathyroidism

As described in Chapter 5, transient and long-term post-operative hypocalcaemia is a significant problem following thyroid and parathyroid surgery which is under-reported.

Length of post-operative stay in hospital is a regularly audited outcome measure of elective surgery. Reduced hospital stay after surgery is a good surrogate marker for reduced complication rates, reducing hospital costs (Khan, Quan et al. 2006).

There is a general trend in thyroid and parathyroid surgery towards a shorter length of post-operative hospital stay (Chadwick, Kinsman et al. 2017). Monitoring and/or treatment of hypocalcaemia is thought to be a cause of increased patient stay after total thyroidectomy (Chadwick, Kinsman et al. 2017). Prevention of hypocalcaemia and management of its symptoms can be addressed pre-operatively, intra-operatively or post-operatively.

Pre-operative optimisation of vitamin D levels may play a role in reducing post-operative hypocalcaemia in patients undergoing total thyroidectomy (Choi, Qeadan et al. 2021) and parathyroid surgery (Rolighed, Rejnmark et al. 2014, Cartwright and Anastasopoulou 2021). The use of pre-operative bisphosphonates may also have a preventative role against post-operative hypocalcaemia in surgery for primary hyperparathyroidism (Lee, Sheu et al. 2006, Mayilvaganan, Vijaya Sarathi et al. 2017). Use of a saturated solution of potassium iodide such as Lugol’s iodine can be
commenced pre-operatively in patients with Graves’ disease to decrease thyroid vascularity and reduce intra-operative bleeding (Yilmaz, Kamer et al. 2016). Both vitamin D optimisation and use of Lugol’s iodine is recommended by the European Thyroid Association and the American Thyroid Association (Ross, Burch et al. 2016, Kahaly, Bartalena et al. 2018). However, the evidence that this improves outcomes, including post-surgical hypoparathyroidism, is poor and therefore uptake of this guidance is not universal. The LIGRADIS trial aims to address this with a multicentre randomised control trial (Muñoz de Nova, Franch-Arcas et al. 2021).

A drive towards more outpatient surgery by the National Health Service in England in 2000 and the addition of thyroidectomy to the list of procedures, approved by the British Association of Day Surgeons has seen a rise in the uptake of day case hemithyroidectomy and parathyroidectomy in selected cases in some UK centres, although this position has not previously been endorsed by the British Association of Endocrine and Thyroid Surgeons (BAETS). Further large published series of day case surgeries have emerged, with data showing that day case thyroidectomy is safe (Bergenfelz, Jansson et al. 2008, Seybt and Terris 2010, Snyder, Hamid et al. 2010, Tuggle, Roman et al. 2011). In 2020, BAETS issued a position statement in support of day case hemithyroidectomy, which is not associated with post-operative hypocalcaemia (BAETS 2020). However, with a drive to have more day case operations, when there is a greater risk of post-operative hypocalcaemia such as total thyroidectomy or parathyroidectomy, robust plans must be in place for its management.

Monitoring and early detection of post-operative hypocalcaemia is important in patients undergoing bilateral or re-do thyroid procedures and parathyroid surgery, so that treatment can be initiated promptly, symptoms avoided, and early discharge facilitated without increasing the potential for re-admission. Whilst BAETS and the Society for Endocrinology provide guidance on post thyroidectomy hypocalcaemia, there is no consensus on post-operative hypocalcaemia management following parathyroidectomy and currently, individual departments are responsible for designing and implementation of their own protocols which will need to be reconsidered if same day discharges are to be initiated. In Sheffield (STHNFT)
protocols are currently in place for those having an inpatient stay as previously stated. The study previously detailed (Chapter 5) has shown that whilst this is fairly robust, there are instances where the protocol is not followed as it does not take into account other factors that may precipitate late hypocalcaemia. These patients may have normal bloods on the first post-operative day but are commenced on supplementation due to the clinician’s judgement that the calcium will continue to fall and render the patient hypocalcaemic in the days following surgery. With same day discharge on the horizon for many hospitals, these protocols will again need to be reassessed. Patients may be given prophylactic regular or pro rata supplementation and followed up with early blood tests to avoid over or under treatment. A consensus on post-operative management of calcium and uniformed guidance is likely to be welcomed but will require tailoring to the services available at each site.

Regular hospital visits place a burden on the patient to attend appointments, including logistics such as parking or dependency on transport in the immediate post-operative period. When designing a protocol for each hospital there needs to be flexibility and the appropriate resources available. The increase in telephone and video consultations and ‘drive through’ phlebotomy services may mean less disruption to the patient than physically having to attend hospital.

6.3 Intra-operative devices

This thesis has investigated two novel technologies which aimed to aid parathyroid identification in neck surgery – near infrared fluorescence and electrical impedance spectroscopy.

Although surgical experience and technique are important in identification and preservation of the parathyroid glands, this is not sufficient in reducing the risk of PoSH to negligible levels.

Given the potential for improvement in surgical outcomes, new technologies have a role to play in improving the ability of the surgeon to identify and preserve parathyroid tissue in central compartment surgery. Identification can be particularly difficult when the thyroid is nodular, when there are reactive or malignant lymph
nodes around the thyroid in re-do surgery, and in bilateral neck explorations when the parathyroids are elusive or in ectopic locations.

Electrical impedance spectroscopy does appear to differentiate the different soft tissues within the neck. However, the Phase IA study conducted as part of this thesis (Chapter 4) demonstrated that further work (in the form of technical modifications to the device and detailed computational modelling of impedance patterns) is required before this technology can be used in a randomised control trial in humans to test whether it improves clinical outcomes by facilitating parathyroid identification and preservation.

Following this feasibility study, further work is ongoing at the University of Sheffield (PhD studentship) that is evaluating the role of advanced computational modelling of the ultrastructure of thyroid and parathyroid cells and evaluating how these models could increase the understanding of impedance patterns of thyroid and parathyroid glands.

Near infrared fluorescence imaging does appear to give the surgeon a wealth of intra-operative information relatively easily. NIRF technologies are becoming more widely available and are often integrated into existing technology such as laparoscopic imaging devices. In neck surgery, the methylene blue fluorescence from the parathyroid gland not only identified the parathyroid gland from the surrounding structures, but it also provided information about the gland’s viability. This technology has the potential to answer the two questions – “is that a parathyroid” and “is it viable?”.

In this study (Chapter 2), patients were excluded with poor renal function or SSRI use. As demonstrated, a large proportion of patients undergoing surgery were not eligible to participate due to these reasons (6.3% not eligible due to eGFR<60, 11.1% taking SSRIs). Going forward, if methylene blue is to be the fluorophore of choice for NIRF in neck surgery then the inclusion of these patients will need to be addressed. The significantly lower dose of methylene blue required for NIRF means that these patients are likely not suffer the neurotoxic side effects seen with high dose methylene blue, but this requires further investigation.
An interesting observation from this study is that parathyroid glands display auto-fluorescence even at 680 nm. Several recent studies investigating auto-fluorescence have established its role in the accurate identification of parathyroid glands (Kahramangil, Dip et al. 2018, Thomas, McWade et al. 2019, Di Marco and Palazzo 2020, Kose, Rudin et al. 2020, Van Slycke, Van Den Heede et al. 2021), enabling its preservation and improving PoSH rates (Benmiloud, Godiris-Petit et al. 2020, Di Marco and Palazzo 2020). These studies however demonstrated auto-fluorescence at much higher wavelengths (Kim, Kim et al. 2018, Serra, Silveira et al. 2019), at which MB fluorescence cannot be relied upon.

In this study and in several other recent reports (Falco, Dip et al. 2016, McWade, Sanders et al. 2016, Kose, Kahramangil et al. 2019, Squires, Shirley et al. 2019, Demarchi, Karenovics et al. 2021), observations were made regarding the difference in auto-fluorescence in normal parathyroid glands and enlarged glands. In parathyroid adenomas, NIRF intensity was found to be heterogenous with the adenomatous part of the gland fluorescing less intensely than the rim of normal parathyroid tissue (Demarchi, Karenovics et al. 2021). Changes in NIRF intensity has also been described in patients with MEN1 where parathyroid auto-fluorescence was decreased and background auto-fluorescence was increased (Squires, Shirley et al. 2019). This was associated with higher rates of both false-negative and false-positive fluorescence. One hypothesis is that patients with MEN1 tend to be younger when they undergo surgery and so have more brown fat. Brown fat, colloidal nodules and metastatic lymph nodes may exhibit auto-fluorescence at a similar wavelength to parathyroid tissue thereby causing false-positive NIRF readings (Demarchi, Karenovics et al. 2020, Demarchi, Karenovics et al. 2021).

Other agents such as ICG could be used with NIRF technology, thus allowing the inclusion of patients with renal impairment or those prescribed SSRIs. Parathyroid auto-fluorescence has been shown to be greatest when excited at a wavelength of 785 nm, re-emitting light at a wavelength between 820-830nm (Demarchi, Karenovics et al. 2020). ICG fluoresces when excited at wavelengths between 750-800nm and re-emits light in the range of 830-845nm. As there is overlap between the ideal excitation and re-emission wavelengths of both parathyroid auto-fluorescence and
ICG, this has become the agent of choice for evaluation in ongoing studies (Demarchi, Karenovics et al. 2020). This is also the fluorophore of choice in other surgeries (e.g., assessing bowel anastomosis perfusion (Blanco-Colino and Espin-Basany 2018)), particularly when investigating the viability of structures.

**6.4 Further work as a result of these studies**

Several improvements have been made to the fluorescent imaging camera following the results described in chapter 2 and from other related studies. This has resulted in the development of the Fluobeam®LX device. A Phase II/III study investigating near infrared fluorescence imaging in bilateral thyroid surgery has been awarded funding by the National Institute for Health Research with Sheffield as the lead centre. The “Near Infrared Fluorescence Imaging to prevent Post-surgical Hypoparathyroidism after Thyroid Surgery” (NIFTy) trial, is a pragmatic, multicentre randomised control trial. The study will investigate the incidence of post-operative hypoparathyroidism at six months, in patients undergoing total or completion thyroidectomy. There will be two groups of patients in this unblinded, parallel group trial – those undergoing surgery using a combination of NIRF with methylene blue and those in the standard arm without MB. Secondary objectives are the incidence of transient hypoparathyroidism, protracted hypoparathyroidism, length of stay in hospital, health related quality of life, readmission rates and hypercalcaemia at six months.

**6.5 Closing comments**

Post-surgical hypoparathyroidism is a significant, but avoidable consequence of neck surgery in most cases. The impact of PoSH on patient’s lives is not well understood but patients can be symptomatic if they are under-treated, within the normal calcium range or as a consequence of treatment. This thesis has shown that there is a significant difference in the reported symptoms and quality of life in patients with PoSH compared to matched controls although standard quality of life measures may not accurately reflect this. Disease-specific quality of life measures may be useful in assessing this particular condition. As new technologies are developed to aid the
intra-operative localisation and assessment of viability of parathyroid glands, one outcome measure must be health reported quality of life (HRQol).

Whilst both near infrared fluorescence and electrical impedance spectroscopy have been shown to differentiate the tissues in the neck, NIRF is the technology that is being developed currently for thyroid and parathyroid surgery. There has been a move away from using methylene blue studied in this thesis, to using ICG as the optimum fluorophore to assess the viability of parathyroid glands. Further clinical trials including the NIFTy trial will assess the effectiveness of this technology in improving clinical outcomes. EIS is a promising tool but requires more improvements in technology and analytical methods before further clinical study.

Operations on the central compartment of the neck other than during thyroid surgery have the potential to cause PoSH. This includes parathyroid surgery as evaluated in this thesis. Further clinical research has the potential to facilitate improvements in the early detection and timely treatment of PoSH following such operations and facilitate reduction in hospital stay, readmission rates and increased patient satisfaction.
References


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neoplasia using electrical impedance spectroscopy with colposcopy.” *BJOG* **120**(4): 400-410; discussion 410-411.


Appendices
Appendix 1: Appendices to Chapter 2

A1.1 Study protocol

TITLE

*Development of a clinical protocol to use intra-operative near infra-red fluorescent imaging in thyroid and parathyroid surgery*

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EudraCT number 2014-001134-28

Study Sponsor: Sheffield Teaching Hospital NHS Foundation Trust
Amendment history

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Protocol_v1.2 – SPB 3rd Dec 2012
Protocol_v1.3 – SPB 9th Jan 2013
Protocol_v6 – SPB 1 Oct 2013
Protocol_v7 – SPB 8 Oct 2013; edited by EW
Protocol_v8 – edited by SPB and EW
Protocol_v9 – edited by SPB 20th Oct 2013
Protocol_v9.1_postSAB – edited by SPB in accordance to SAB comments
Protocol_v10 – further edits by SPB
Protocol_v11 – edits by EW & SPB
Protocol_v14 - edits by JC & SPB as requested by MHRA
1. SUMMARY

Around 13,000 thyroid and parathyroid operations are performed per year for both benign and malignant disease. Reliable identification of parathyroid glands is critical to the success of thyroid and parathyroid surgery. In thyroid surgery, inadvertent injury to parathyroid glands may cause temporary or permanent hypoparathyroidism (low calcium levels needing long term treatment); the latter is associated with significant long-term problems. In parathyroid surgery, early identification of normal and/or enlarged parathyroid glands helps in deciding on the extent of surgery and increases the chances of postoperative normal calcium levels.

Methylene Blue (MB, Methylthioninium chloride) is a dye that when given intravenously in high doses, is taken up differently by thyroid and parathyroid tissue. It is currently used during parathyroid surgery by some surgeons to help identify enlarged parathyroid glands by visual examination alone. At these doses, there is a risk of adverse effects from administration of MB. Such visual examination is unhelpful in the identification of ‘normal’ parathyroid tissue. MB is not currently used in surgery for thyroid pathologies. MB exhibits fluorescent properties in the near-infrared range (light just beyond the visible spectrum). This can be picked up by an appropriate imaging system. This has the potential to identify and differentiate between ‘normal’ parathyroid, ‘abnormal’ parathyroid and thyroid tissue during surgery.

We have established the feasibility of the intra-operative use of a near infra-red fluorescent imaging device called Fluobeam® and demonstrated the ability of this device to pick up near infra-red fluorescence from human tissue after administration of intravenous MB. Animal experiments have shown that doses as low as 0.1mg/kg of MB given intravenously enable fluorescent visualisation of thyroid and parathyroid glands.

This study will aim to optimise the dose and timing of administration of MB in human thyroid and parathyroid surgery and to develop a protocol which would then subsequently be assessed for effectiveness in a multi-centred randomized controlled setting.
2. BACKGROUND

An important adverse effect of thyroid surgery (particularly total thyroidectomy) is transient or permanent hypoparathyroidism with resulting hypocalcaemia. The mechanisms of parathyroid insufficiency following thyroidectomy include inadvertent parathyroidectomy, gland devascularisation and obstruction of venous drainage (Sitges-Serra, Ruiz et al. 2010). Inadvertent excision of the parathyroid gland is not uncommon in patients undergoing thyroidectomy (Sakorafas, Stafyla et al. 2005, Sorgato, Pennelli et al. 2009) and is thought to be largely due to non-visualisation of the glands during surgery. Permanent hypoparathyroidism not only causes debilitating symptoms that requires long term treatment but is also associated with other significant morbidity including renal stones, nephrocalcinosis and soft tissue calcification (Khan, Waguespack et al. 2010).

In thyroid surgery, individual centres have shown very low (up to 1.4%) rates of permanent hypoparathyroidism (Pattou, Combe male et al. 1998, Pisanu, Piu et al. 2005, Page and Strunski 2007, Youngwirth, Benavidez et al. 2010) following total or subtotal resection. However, figures published by a Scandinavian multicentre audit show 'self-reported' rates of at least 4.4% (Bergenfelz, Jansson et al. 2008). In the UK, the British Association of Endocrine and Thyroid Surgeons (BAETS) fourth national audit reported a 25% and 12% incidence of transient and long term hypocalcaemia following total thyroidectomy (Chadwick and Kinsman 2012). The limitations of the audit figures are that there is a significant proportion of missing data; the data is ‘self-reported’, and the audit only represents around a third of all thyroid surgery in the UK. Any technique aimed to facilitate the accurate and consistent identification of normal parathyroid glands during thyroidectomy has the potential to reduce the incidence and severity of hypoparathyroidism and thereby the morbidity of surgery.

In parathyroid surgery, the identification of normal/enlarged parathyroid glands during surgery for primary/renal hyperparathyroidism and the intra operative differentiation between normal and enlarged glands is crucial to the success of the procedure. Although this is often straightforward in patients with single gland disease; the detection and appropriate treatment of patients with multi gland disease
(accounting for 10-15% of patients with primary hyperparathyroidism and all patients with renal hyperparathyroidism) can be challenging as demonstrated by the increased incidence of persistent/recurrent disease in this subset of patients (2009). Preoperative or intra-operative identification of a parathyroid adenoma is currently facilitated by the use of varying combinations of preoperative (Chien and Jacene 2010) and intra-operative techniques (Harrison and Triponez 2009) including ultrasound, MIBI scan, frozen section, gamma probe, intra-op PTH assay and Methylthioninium chloride (MB). MB, when used in doses of up to 7.5mg/kg body weight helps in visualisation of the vast majority of abnormal parathyroid glands. These doses are associated with temporary urine discoloration, pseudocyanosis and pseudohypoxia. Adverse events include nausea, pain in infused arm, local oedema/thrombophlebitis, transient hypotension and angina. Rare but serious neurological side effects that are potentially life threatening (especially in patients on concomitant serotonin re-uptake inhibitors) have also been reported (Patel, Chadwick et al. 2012). Moreover, the identification of ‘normal’ parathyroid glands, which is sometimes required to rule out multi gland disease, is facilitated neither by MB nor by any of the above techniques.

Intra-operative near infrared fluorescent imaging has found application in many different surgical procedures (Gioux, Choi et al. 2010) as a tool for the identification and differentiation of tissues with varying ability to take up and retain fluorescent dyes. Methylene Blue (MB), when given intravenously is known to be differentially taken up by thyroid, ‘normal’ parathyroid and ‘abnormal parathyroid tissue and been used in the ‘naked-eye’ identification of an enlarged (adenomatous/hyperplasic) parathyroid gland for some time. However, visual examination with intravenous MB does not help in identification of ‘normal’ parathyroid glands. MB is known to have near infrared fluorescence properties which have potential for use in surgery (Matsui, Tanaka et al. 2010, Matsui, Tanaka et al. 2010). This property could be used to facilitate the detection of normal and enlarged parathyroid tissue after intravenous administration of MB. The identification of ‘normal’ parathyroid glands would help in their preservation during thyroid surgery and reduce the risk of postoperative hypocalcaemia. In parathyroid surgery, early identification of ‘enlarged’
parathyroid glands has the potential to reduce operating times and the identification of ‘normal’ parathyroid glands would help in ruling out multi-gland disease.

We postulate that the use of a fluorescent imaging system to detect MB emitted near infrared fluorescence will aid in the identification of normal and abnormal parathyroid tissue. This has the potential to improve outcomes in both thyroid and parathyroid surgery. We have performed a phase I feasibility study using an ‘intra-operative near infra-red fluorescent imaging’ technology (Fluobeam® developed by Fluoptics) and intravenous MB during parathyroid surgery. This study (Balasubramanian, Lee et al. 2013) has shown that MB fluorescence can be observed during neck surgery and there are differences in fluorescence between thyroid, parathyroid and other soft tissue structures in the neck. Use of Fluobeam® during surgery was ergonomically feasible and did not hamper the course of the operation.

We have also demonstrated in an animal model (Antakia, Gayet et al. 2013) that doses as low as 0.1mg/kg of MB administered intravenously enables detection of MB fluorescence in neck endocrine glands. These demonstrated that thyroid tissue fluoresces more intensely than parathyroid tissue; time of onset of fluorescence was similar in thyroid and parathyroid glands; the average time to peak parathyroid fluorescence (up to 3 minutes) was shorter than the time to peak thyroid fluorescence (up to 8 minutes); and the washout of parathyroid fluorescence was relatively quicker than thyroid (see Figure 1). These properties demonstrate the potential of this technology to aid differentiation between these tissues at surgery. If valid in humans, this will lead to the use of approximately 1/30th of dose currently used resulting in a much improved safety profile for Methylthioninium chloride.

The Phase Ib study will include patients undergoing thyroid and parathyroid surgery and will evaluate the optimum dose and timing of administration of MB and duration of staining of thyroid and parathyroid tissue as visualised using ‘Fluobeam®’. This will enable development of a protocol for the use of Fluobeam® and intravenous MB in routine thyroid and parathyroid surgery.
Further research will include a randomized controlled trial to evaluate the effectiveness of near-infrared fluorescent technology in improving outcomes following thyroid and parathyroid surgery.
Figure 1. Chart illustrating changes in fluorescence intensities from thyroid, parathyroid and muscle in a rabbit following injection of different doses of intravenous Methylthioninium chloride. The x-axis plots the time following exposure of the central neck compartment. The y-axis shows fluorescent intensity as measured by Image J software.

The times shown in the figures adjacent to the peaks refer to the time to highest peak fluorescence in thyroid (purple) and external parathyroid glands (black).
3. HYPOTHESIS, AIMS AND OBJECTIVES

Hypothesis: Intra-operative use of near infrared fluorescent imaging technology and detection of Methylthioninium chloride fluorescence will facilitate early and accurate identification of parathyroid glands (both normal and abnormal) and thereby improve outcomes following thyroid and parathyroid surgery.

Aim: The aim of this phase is to develop a protocol for the use of Fluobeam® with intravenous MB in the detection of parathyroid tissue and its differentiation from adjacent soft tissue during surgery on thyroid and parathyroid glands.

Objectives:

To identify the optimum dose of intravenous MB and time to peak fluorescence of normal and abnormal thyroid and parathyroid glands.

To determine differences in patterns (onset, intensity and duration) of fluorescent staining between the various soft tissues of interest in the neck.
4. METHODS

The project requires Ethical, MHRA and R and D approval.

Study Design

This is an interventional study, without a control arm. Two groups of patients will be included. The first will be patients undergoing bilateral neck exploration (BNE) for primary hyperparathyroidism (PHPT) and the second will include patients undergoing total thyroidectomy. Both groups of patients are required as uptake and retention of Methylthioninium chloride (MB) may be different in different diseases of the thyroid and parathyroid glands and as there is potential for the protocol to be used in both clinical settings.

The study is divided in three stages; referred to as the ‘training’, ‘testing’ and ‘final’ stages.

Training stage

The aim of this stage is to estimate the optimal dose of MB and the time of observation and develop a working protocol. Around twenty patients (10 in each of the thyroid and parathyroid surgery groups) will be included in this stage.

During surgery (under anaesthesia) after exposure of the thyroid and/or parathyroid gland(s) in the central compartment, boluses of between 0.05 to 0.5mg/kg of Methylthioninium chloride will be injected. Fluobeam® (handheld fluorescence reflectance real time imager) will be used to record still/video images of the operating field from just before the start of the injection to up to 20 minutes after injection. If the parathyroid glands on both sides are not clearly visible in the same field of vision, only one side (to be decided by the surgeon) will be used for measurements.

For the first patient, a dose of 0.05mg/kg body weight of MB will be administered. In rabbit studies, a dose as low as 0.1mg/kg of MB was found to demonstrate fluorescence effectively. On this basis, a lower starting dose in human experimentation is considered reasonable. If no staining is recorded at up to 20
minutes, a further bolus of 0.1mg/kg will be infused. The dose and timing of MB infusion to be used in the first patient is presented as a table below.

<table>
<thead>
<tr>
<th>Protocol for use in the first patient in the study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strength of MB</strong></td>
</tr>
<tr>
<td>First dose</td>
</tr>
<tr>
<td>Second dose</td>
</tr>
<tr>
<td>Maximum total dose / duration</td>
</tr>
</tbody>
</table>

Based on findings in the first patient, the volume of MB for the first dose in subsequent patients will be decided. The strength of second dose will be decided based on the response to first dose. If the patient experiences significant intolerance e.g., cardiac arrhythmia or significant hypotension in response to the first dose (bolus) then a second dose will not be administered. The change in dosage is expected to be in increments or decrements of 0.025 to 0.05mg/kg as a ‘titrate to effect’ dose until ‘satisfactory’ fluorescence from thyroid and parathyroid glands are detected. The level of fluorescence considered ‘satisfactory’ will be based on a subjective assessment by the investigator in conjunction with the operating team based on observation of images visualized using Fluobeam®.

To limit time under anaesthesia, a maximum of two boluses will be used for each patient and the total time of observation will be a maximum of 30 minutes. A total of not more than 1.0mg/kg body weight of Methylthioninium chloride will be administered to each patient; although most patients are likely to receive much less
than this. This is one-third of the maximum dose that was being used until recently in Sheffield for parathyroid surgery and is much lower than doses of 5-10 mg/kg used elsewhere (MHRA 2008). Following the observation period, the operation will be carried out in the standard manner and not be influenced by any observations made by fluorescent imaging. The use of MB to guide parathyroid surgery is only valid at currently used higher doses and MB is not used in thyroid surgery currently.

Fluorescent intensity from the various soft tissue structures can be measured (using ‘Image J’ software) by marking the area of interest in the images recorded. The changes in fluorescence in thyroid lobes, parathyroid glands and skeletal muscle over time for the different doses studied will be recorded. This data will be reviewed and (as previously mentioned) used as the basis for the first dose and duration of observation for subsequent procedures. It is expected that the procedure will be repeated at same dose in at least two to three patients to ensure that any inter-patient variation is understood before a change is considered. This will help determine the minimum dose required for the detection of near infrared fluorescence from MB in the neck soft tissues. This may be different for patients undergoing thyroid and parathyroid surgery. The protocol will be developed in parallel for patients undergoing thyroid and parathyroid surgery as there may be a difference in staining between normal and abnormal thyroid and parathyroid glands. Data from both sets of patients will however be used to refine the protocol as the study progresses. This is because patients undergoing parathyroid surgery often have some normal parathyroid glands and patients undergoing thyroid surgery often have a significant amount of normal background thyroid tissue.

An example of a decision tree (to enable decisions on first dose of Methylthioninium chloride in subsequent patients) is shown below.
Observations on fluorescent intensity on images recorded at different times during surgery will help determine the time of ‘peak fluorescence’ from the various soft tissue structures and the time of maximum differential staining between the parathyroid gland and surrounding tissues. The determination of optimal dose and time of assessment will require several iterations of dose /observation time combinations. As these experiments will be carried out in patients with a range of thyroid and parathyroid diseases, any variation in MB uptake and fluorescence due to disease pathology will also be examined, although the study is not powered to detect significant differences between groups.

**Testing stage**

The aim of this stage is to test and further optimise the working protocol that would have been generated at the end of the training stage. Only a single bolus of MB will be used in patients in this stage of the study.

This protocol may be different in the two groups of patients (i.e., those undergoing thyroid and parathyroid surgery). A sample size of up to 15 patients for this phase allows the working protocol to be revised (with different doses of MB) if it becomes clear there are major deficiencies. Any changes to the doses of MB at this stage is expected to be in increments or decrements of 0.05mg/kg. Although it is difficult to
establish clear criteria to define an optimal protocol, the following guidelines are proposed to help decide on when a protocol may be considered successful in this stage.

**Thyroid surgery**

A consistent (with up to 20% variation) peak fluorescent intensity ratio (parathyroid to muscle peak intensity) is obtained from at least one parathyroid gland in three consecutive patients using a specific dose AND

The range of ‘time to peak fluorescence’ for the above glands is less than ten minutes.

**Parathyroid surgery**

A consistent (with up to 20% variation) peak fluorescent intensity ratio (thyroid to muscle peak intensity) is obtained from thyroid tissue in three consecutive patients using a specific dose AND

The range of ‘time to peak fluorescence’ for the above glands is less than 10 minutes.

**Final stage**

The aim of this stage is to enable collection of preliminary data using the protocol considered to be optimum at the end of the testing stage. The initial efficacy data generated in this stage will be used to plan the eventual trial. Only a single bolus of MB will be used in patients in this stage of the study. The total number of patients to be studied in this stage is 15 (up to 7-8 in each group).

The end point of the trial will be the development of a protocol that can be implemented in patients undergoing thyroid and parathyroid surgery to enable early and accurate identification of parathyroid glands.

**IMP preparation**

0.5% w/v Methylene Blue (Provepharm’s Methylene Blue Injection EP, “Methylthioninium chloride Proveblue” 5 mg/mL, Solution for injection) will be used
in this study. This is supplied as 10 ml ampoules which contain 50mg Methylthioninium chloride per ampoule. Supplies will be kept in pharmacy and dispensed labelled for clinical trial use. MB will be given undiluted as a slow bolus. For low volumes of MB, the cannula will be flushed with an appropriate intravenous solution that is already being infused as part of standard treatment. Records of the dose of Methylthioninium chloride administered will be kept in the case report form. Unused Methylthioninium chloride will be discarded.

Fluorescent Imaging System: ‘Fluobeam®’ is a technology developed by Fluoptics, a commercial company based in Grenoble, France. This is a portable, handheld fluorescence reflectance real time imager. The field of view is illuminated with a high quality ambient light; the illumination source for the excitation being is provided by a Class 1 laser. The fluorescent light emitted by the fluorophore (Methylthioninium chloride) under NIR laser excitation is collected and projected on a remote screen with a maximal spatial resolution of 70μm.

The Fluobeam® device will only be used during the period of observation after intravenous injection of MB. The system is CE marked and does not pose any direct risk to the patient. The chief investigator is familiar with the workings of the device and system and will train a co-investigator to operate the system.

The Fluobeam® device currently costs around £59,000. Costs are likely to reduce with increasing use. The phase I study has confirmed that the device does not require any additional infrastructure or equipment for its application. The device is covered in a sterile, plastic cover before being used in patients. This cover is disposable and so will not pose an infection risk to patients. The device can be transported to and between different operating theatres with ease. There are no anticipated problems that could hinder dissemination or widespread use of the device in hospitals across the NHS.

Eligibility

Inclusion criteria:
All patients undergoing either a total thyroidectomy or bilateral neck exploration for PHPT in Sheffield Teaching Hospitals NHS Foundation Trust.

Patients aged 18 years and older.

**Exclusion criteria:**

patients undergoing re-do procedures,

patients unable to understand spoken and written English,

patients unable to give adequate informed consent,

patients with a history of intolerance or sensitivity to MB,

patients with known G6PD deficiency.

patients on serotonin reuptake inhibitors or taking any medicinal products that enhance serotonergic transmission including for example SSRIs, bupropion, buspirone, clomipramine, mirtazapine and venlafaxine.

patients undergoing surgery for thyroglossal cyst and

patients undergoing thoracic exploration; either alone or in combination with a neck exploration.

Patients who are pregnant or breastfeeding.

Patients with an eGFR of ≤ 59 ml/kg/1.73m² (eGFR is checked as part of routine work up before surgery and the last available measurement will be used to make this decision)

Eligibility will be formally confirmed by the investigator before IMP is administered to the patient.

**Recruitment strategy**

Patients undergoing thyroid and parathyroid surgery for both benign and malignant disease in Sheffield Teaching Hospitals are managed in the Endocrine Surgical unit of
the Directorate of General Surgery. Patients suitable for inclusion in the study will be identified by clinicians and approached at their visit to hospital. This may either be at the endocrine surgical clinic (three clinics a week – Monday, Tuesday and Friday AM) or the pre-assessment unit at the Royal Hallamshire Hospital, Sheffield. Information about the study will be presented and those expressing an interest in the study will be seen by a member of the research team who will explain the study protocol in detail. An information sheet and consent form will be provided. All patients willing to take part in the study will be requested to sign the consent form in the presence of an appropriate member of the research team, who will ensure that the patients are fully informed of the implications of taking part in the study. Patients will not be required to indicate their willingness to participate on the same day. They will be approached at their next visit to hospital (such as the pre-assessment clinic or on the day of surgery) and those that consent to take part will then be included in the study. Any further questions or clarifications will be addressed either by telephone conversation or during subsequent visits to hospital. Any patient wishing to withdraw from the study at any stage following initial inclusion will be free to do so and all data relating to the patient will be deleted from the research database.

Female patients (excluding those over 55 and those who are more than 2 years since the last menstrual period) are routinely tested for pregnancy on a urine sample using a ‘near patient’ testing system. Patients who are pregnant will be excluded from the study.

Data collection and management

Upon receipt of written, informed consent the study investigator will access the medical records and extract information relating to demographics (weight, gender) and clinical/pathological details (such as indication for surgery). The patient may be approached to clarify or fill in uncertain or missing information. Information relating to use of MB (whether it was actually used, dose, number of doses, adverse effects): information on any adverse events from MB injection (such as extravasation, hypotension within 30 minutes of use, other adverse events thought by the anaesthetist to be linked to MB); intra-operative details (whether Fluobeam® was
used, time of incision and closure, time at which observation was concluded and intra-operative findings); and postoperative information (on adverse events, histology and symptoms relating to hypocalcaemia) will be recorded. All of the above information will be recorded on the study case report forms (see attached).

Observations on the fluorescence will be recorded directly on the computer linked to the Fluobeam® device. Colour pictures of the operating field may also be taken during the period of observation to enable correct identification of soft tissue structures and correlation with the black and white/fluorescent images taken by the device. From this data, the time of onset and time to peak fluorescence of the various glandular structures in the operating field will be recorded on the case report forms. The date on which the data on the computer was analysed for each patient and the decision regarding the starting dose in subsequent patients will also be recorded.

All information will be maintained as hard copies (and stored in the office space of the Department of Oncology at the University of Sheffield) and/or electronically on a secure database.

Data monitoring group

A data monitoring group has been formed. Two independent local academics (Ms Lynda Wyld and Prof John Newell-Price) and an external endocrine surgeon (Mr David Chadwick) have agreed to be part of the data monitoring group. The group will be convened once before the start of the study. They will be asked to review the data at least every three months and prior to the start of each stage. The group members will be asked to report on the validity of the investigators’ judgements regarding changes to MB dose, duration of observation and the working protocol.

Statistical considerations

The design and statistical considerations have been discussed with Dr Mike Bradburn, statistician at the Clinical Trials Research Unit in SCHARR at the University of Sheffield. There are no formal statistical grounds (e.g., precision or power) on which to inform a sample size. A sample size of 50 (25 of each procedure) has been selected based on the pragmatic grounds of a “training” cohort (10 in each group, 20
patients in total), followed by a “test” cohort (7-8 in each group, 15 patients in total) in which the procedure is refined, and a “final” cohort (7-8 in each group, 15 patients in total) in which the working protocol is adopted. A formal data review will be undertaken by the chief investigator in conjunction with other members of the research team with the peri-operative data available on the training cohort patients. In the training stage, a working protocol (with particular reference to the dose) to be used for patients in the “testing” stage will be developed. The data monitoring group will review the data at this stage and validate the working protocol.

The definition of ‘normal’ and ‘abnormal’ gland tissue will be made by the investigators taking in consideration the following factors: the clinical diagnosis, intra-operative and histology findings where applicable and normalization of biochemistry (in case of parathyroid glands).

Descriptive analyses will include summaries of peak fluorescence ratios and time to peak fluorescence of thyroid and parathyroid glands; overall and stratified by dose of MB and ‘normal’ versus ‘abnormal’ nature of the glands. The data will be described using mean (+SD) for continuous data that is normally distributed; and median (+range) for ordinal data or continuous data that is not normally distributed. Any adverse outcomes (overall and by relationship to the intervention) will be tabulated.

Comparisons between ‘normal’ thyroid and parathyroid peak fluorescence ratios and time to peak fluorescence will be made using the paired T test or Wilcoxon rank test as appropriate. This analysis will be done individually for those strengths of MB given as first dose, provided adequate number of patients with measurements on normal glands are available. Further exploratory analyses may be made comparing fluorescence from the first detected abnormal and normal parathyroid glands in independent patients who have been subjected to the same protocol of administration of Methylthioninium chloride; provided the numbers are adequate. The statistical method to be used for this analysis will be the Student T test or Mann Whitney U test as appropriate.

Other outcomes
Intra-operative adverse events directly related to Methylthioninium chloride injection such as extravasation of MB and intra-operative hypotension within 30 minutes of injection will be recorded. At the first postoperative visit (between one and 12 weeks of surgery), further data will be collected on each patient. This will include

Adjusted calcium levels, symptoms of hypocalcaemia and need for calcium/vitamin D supplements for hypocalcaemia.

Histology results - to confirm suspected pathology and correlate with intra-operative findings. This will also enable an assessment of whether pathology significantly influences fluorescent signals from MB uptake and retention.

Serious adverse events and SUSARs will also be recorded.

Adverse Events

Data on all serious adverse events occurring in this trial will be collected and reported immediately to the sponsor.

As Methylthioninium chloride is a licensed, well-tested medication we will only collect data in this trial on non-serious adverse events that are thought to be related to MB which occur intra-operatively or in the 24 hours after dosing. Data on non-serious adverse events in the postoperative period after 24 hours post dose will not be collected. Data on adverse events which are not related to Methylthioninium chloride will not be recorded.

As hypotension and cardiac arrhythmia are potential side effects of MB, the patient will be observed for the occurrence of these problems by continuous cardiac (ECG) monitoring and blood pressure measurements. This is standard care for patients undergoing general anaesthesia. Significant events will be captured on the adverse event forms.

A Serious Adverse Event (SAE) is any adverse event, regardless of the event’s relationship to the IMP which results in the following outcomes:

1. Death
2. A life-threatening adverse event

3. Inpatient hospitalisation or prolongation of existing hospitalisation

4. A disability / incapacity

5. A congenital anomaly in the offspring of a participant

The reporting period for Serious Adverse Events will be from the time of induction of anaesthetic until 30 days after the day of surgery. Important medical events that may not result in death, be life-threatening, or require hospitalisation may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. All adverse events will be assessed for seriousness, expectedness and causality:

The investigator will determine seriousness as per the criteria above.

The investigator will report all serious adverse events (SAEs) to the study sponsor within 24 hours of learning of the event irrespective of the causality of the event. The investigator will make the report by faxing a completed SAE form to STH Research Department.

Causality

Unrelated or improbable: a clinical event with temporal relationship to trial treatment administration, that makes a causal relationship incompatible or for which other drugs, chemicals or disease provide a plausible explanation. This will be counted as “unrelated” for notification purposes.

Possible: a clinical event with temporal relationship to trial treatment administration which makes a causal relationship a reasonable possibility, but which could also be explained by other drugs, chemicals or concurrent disease. This will be counted as “related” for notification purposes.

Probable: a clinical event with temporal relationship to trial treatment administration which makes a causal relationship a reasonable possibility, and is unlikely to be due
to other drugs, chemicals or concurrent disease. This will be counted as “related” for notification purposes.

Definite: a clinical event with temporal relationship to trial treatment administration which makes a causal relationship a reasonable possibility, and which can definitely not be attributed to other causes. This will be counted as “related” for notification purposes.

An AE whose causal relationship to the study IMP is assessed by the Principal Investigator as “possible” “probable” or “definite” is an Adverse Drug Reaction.

Expectedness

Known adverse reactions to Methylthioninium chloride, as described in the current Summary of Product Characteristics will be considered as ‘expected’

Suspected Unexpected Serious Adverse Reaction

A SUSAR is any event which qualifies as an SAE and meets the criteria of being judged as possibly, probably or definitely related to study medication and has a ‘nature and/or severity’ which is not consistent with the information about the medicinal product in question as set out in the summary of product characteristics for that product’. It is the responsibility of the Principal Investigator to classify each SAE with regard to causality and expectedness and to report this classification to the study sponsor within 24 hours of the classification being determined. Where a SUSAR is identified STH Research Department will be responsible for reporting to the Regulatory Authorities as per the clinical trials regulations.

Potential/anticipated problems:

Currently, intravenous high dose Methylthioninium chloride is not routinely used during thyroid and/or parathyroid surgery in the endocrine surgical unit of Sheffield Teaching Hospitals NHS Foundation Trust. The use of MB during surgery is proposed to be at very low doses and side effects are expected to be negligible. This will however be closely monitored during the study.
There is potential for other tissue to be detected with the device and mistaken for parathyroid tissue. The surgeons will ensure that fluorescence detection will not be a criterion for use in decision making and that other currently used features such as findings on preoperative imaging, location, size, colour and consistency will be used for management purposes.

For safety reasons, it may not be possible to ensure uninterrupted recording of observations after injection of MB. This will be dealt with in a pragmatic manner with patient safety as the key priority. The design of the experiment allowing repetition of the experiments in subsequent patients will address this problem.

The investigators will ensure that patients who do not consent to the procedure receive standard treatment as planned.

These numbers quoted in the various phases of the study are conservative since experiences gained from thyroid surgery will inform parathyroid surgery and vice versa. The surgeons in Sheffield perform around 250 thyroid and/or parathyroid surgeries a year at Sheffield Teaching Hospitals and are well placed to recruit the required number of patients for this study.

The gold standard for accurate identification of thyroid and parathyroid glands (based on which fluorescent measurements will be made) will be the surgeon’s localisation and judgement. Although this is straightforward for thyroid glands, it may not be accurate for parathyroid glands. This will be checked by subsequent histology if the glands are removed. For this reason, only those parathyroid glands that are identified with ‘certainty’ by the surgeon will be used for the observations.

**Ethics and good clinical practice (GCP)**

The study will only begin after being approved by the Medicines and Healthcare Regulatory Agency (MHRA) and the Regional Research Ethics Committee (REC). The study will be performed in accordance with recommendations guiding physicians in biomedical research involving human subjects (Declaration of Helsinki). Informed written consent will be obtained from all patients prior to entry into the study. The right of the patient to refuse participation without giving reason will be respected.
and patients will remain free to withdraw their consent for the storage of data in the study at any time without giving reasons and without prejudicing future treatment. Any amendment to the protocol will only be made with the approval of the chief investigator and will be subject to review by the REC. The trial will be registered with a trials registry (www.clinicaltrials.gov).

The members of the research team will have attended the good clinical practice (GCP) training by the time of the start of the study.

Confidentiality

The study team will be responsible for recording findings and collecting data in both phases of the trial. Summary data obtained from this study will be used to design the third phase. All aspects of the Data Protection Act 1998 will be complied with. Any information that would allow patients and clinicians (who are not part of the research team) to be identified will not be released into the public domain.

Archiving

Data relating to the trial will be securely archived for a minimum of 15 years. If a patient withdraws consent for their data to be used the material will be confidentially destroyed.

Indemnity

As the study will be carried out by university staff (with honorary contracts with the hospital) and by STH employees, both NHS indemnity and University insurance will apply as per the standard protocols. Manufacturer product liability will also apply as the drug (Methylthioninium chloride) will be used outside the strict terms of the Marketing Authorisation. Indemnity for equipment will also be in place as the research will be conducted using equipment from a commercial company (Fluoptics).
A1.2 Ethical Approval

Health Research Authority
National Research Ethics Service
NRES Committee North West - Greater Manchester Central
3rd Floor
Barlow House
4 Minshull Street
Manchester
M1 3DZ
Telephone: 0161 625 7825
Fax: 0161 625 7289

23 June 2014

Mr Saba Balasubramanian
University of Sheffield
EU35, E Floor,
Royal Hallamshire Hospital
Sheffield
S10 2JF

Dear Mr Balasubramanian

Study title: Development of a clinical protocol to use intra-operative near infra-red fluorescent imaging in thyroid and parathyroid surgery

REC reference: 14/NW/0270
Protocol number: STH17176
EudraCT number: 2014-001134-28
IRAS project ID: 145543

Thank you for your letter responding to the Committee’s request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Mrs Kath Osborne, nrescommittee.northwest-gmcentral@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised subject to the conditions specified below.
Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which can be made available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission (“R&D approval”) should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at [http://www.rdforum.nhs.uk](http://www.rdforum.nhs.uk).

Where a NHS organisation’s role in the study is limited to identifying and referring potential participants to research sites (“participant identification centre”), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publicly accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made.

Guidance on where to register is provided within IRAS.

Clinical trial authorisation must be obtained from the Medicines and Healthcare products Regulatory Agency (MHRA).

The sponsor is asked to provide the Committee with a copy of the notice from the MHRA, either confirming clinical trial authorisation or giving grounds for non-acceptance, as soon as this is available.

A Research Ethics Committee established by the Health Research Authority
It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

**Ethical review of research sites**

**NHS sites**

The favourable opinion applies to all NHS sites listed in the application, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

**Approved documents**

The final list of documents reviewed and approved by the Committee is as follows:

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<th>Version</th>
<th>Date</th>
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**Statement of compliance**

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

**After ethical review**

**Reporting requirements**

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol

A Research Ethics Committee established by the Health Research Authority
• Progress and safety reports
• Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

We are pleased to welcome researchers and R & D staff at our NRES committee members’ training days – see details at http://www.hra.nhs.uk/hra-training/

14/NW/0270 Please quote this number on all correspondence

With the Committee’s best wishes for the success of this project.

Yours sincerely

K. Osborne

Signed on behalf of
Professor S J Mitchell
Chair

Email:nrescommittee.northwest-gmcentral@nhs.net

Enclosures: “After ethical review – guidance for researchers”

Copy to: Dr E Wallis, Research Department.
Ms J Clarke
SHEFFIELD TEACHING HOSPITALS NHS FOUNDATION TRUST
SHEFFIELD TEACHING HOSPITALS (STH), RESEARCH DEPARTMENT
11 BROOMFIELD ROAD
SHEFFIELD
S10 2SE
UNITED KINGDOM

29/07/2014

Dear Ms J Clarke

THE MEDICINES FOR HUMAN USE (CLINICAL TRIALS) REGULATIONS 2004 S.I. 2004/1031

Our Reference: 21304/2052/001-0001
EudraCT Number: 2014-001134-28
Product: Methylthionium chloride Proveblue
Protocol number: STH17176

NOTICE OF ACCEPTANCE OF AMENDED REQUEST

I am writing to inform you that the Licensing Authority accepts your amended request for a clinical trial authorisation (CTA), received on 29/07/2014.

The authorisation is effective from the date of this letter although your trial may be suspended or terminated at any time by the Licensing Authority in accordance with regulation 31. You must notify the Licensing Authority within 90 days of the trial ending.

Finally, you are reminded that a favourable opinion from the Ethics Committee is also required before this trial can proceed; changes made as part of your amended request may need to be notified to the Ethics Committee.

Yours sincerely,

Clinical Trials Unit
MHRA
Participant information sheet (Parathyroid)

Development of a clinical protocol to use near infra-red fluorescent imaging during thyroid and parathyroid surgery

We would like to invite you to take part in a research study. Before you decide to take part, it is important to understand why the research is being done and what is involved. This will help you make an informed decision. Please be reassured that your clinical care will not be affected by your decision.

One of our team will go through the information sheet with you and answer any questions you may have. This should take around 15 to 30 minutes. Please take time to decide and discuss it with others if you wish. Please ask us if there is anything that is not clear or if you would like more information.

Background to the study

The parathyroid glands are four tiny pieces of tissue. Each is about the size of a grain of rice. These glands are situated on either side of the thyroid gland in the neck. They produce a hormone called PTH (Parathyroid Hormone). PTH helps maintain blood calcium levels. Without parathyroid glands, the blood calcium level would fall. This can produce a range of unpleasant symptoms including tingling, pins and needles, fatigue, painful muscle cramping and if left untreated seizures. There is also potential for long term damage to the kidneys and bones.

During parathyroid surgery, it is important to identify parathyroid glands and to differentiate between ‘normal’ and ‘abnormal’ glands. This will improve the success rates and reduce risk of damage to normal parathyroid glands. Currently available techniques (such as ultrasound and MIBI) focus only on identifying enlarged parathyroid glands and do not help to identify normal parathyroid glands.
This study aims to evaluate a new technique in the identification of both normal and abnormal parathyroid glands during parathyroid and thyroid surgery. If successful, the technique has potential to improve outcomes from these operations.

What do we do in this study?

A dye (methylene blue) that tends to accumulate in parathyroid glands is occasionally injected into the vein prior to surgery to help identify the abnormal parathyroid glands. In this study, this dye will be injected during the operation at much lower doses than currently used. In some instances, a second dose may be injected.

This dye has special (fluorescent) properties, which will be picked up by a recently developed fluorescent imaging system (Fluobeam®). The brightness of fluorescence is different for the different tissues/organs in the neck and will vary with time. This may also vary between normal and abnormal parathyroid glands.

Recordings using Fluobeam® (and digital pictures) will be taken following the injections for a specified period of time during surgery. We hope to use the findings from the study to determine if the equipment will help the surgeon in identifying parathyroid tissue and help differentiate between normal and abnormal parathyroid tissue.

What are the possible benefits of taking part?

You will not directly benefit by taking part in this study. If the technology appears to work, it will be further tested in another clinical trial to see if the risk of complications following thyroid surgery and the rates of failure following parathyroid surgery can be lowered. This research therefore has potential to benefit patients undergoing thyroid or parathyroid surgery in the future.

What are the risks of taking part?

There is a small risk of allergic reactions from the dye and irritation or damage to the skin at the site of infusion. These problems are however very rare and can be treated if they arise.

The fluorescent imaging technology used during the operation to make recordings is unlikely to influence the nature of the operation or the outcomes.
There will however be some increase in the duration of the operation due to the need to make the observations. The maximum period of observation has been fixed at 10 minutes.

**Do I have to take part?**

No. Taking part is completely voluntary. It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form. However, you are free to withdraw at any time. Whether you take part or not will not affect the standard of care you receive in any way at any time.

Obtaining informed consent for your planned operation is a separate process which will occur in parallel to the consent process for this research. This will be done by a member of the surgical team responsible for your care.

**What will happen to me if I decide to take part?**

You will have a discussion with a member of the research team. They will go through the details of the study and answer any questions you may have. You will then be asked to sign a consent form informing us that you agree to be involved. Three copies of this form will be made, of which one copy is for your records. You will have your surgery as scheduled. During the surgery you will have the dye injection, following which the surgery will pause for a few minutes whilst the fluorescent imaging device is used to observe the thyroid and parathyroid area. In some cases a second injection and a further period of observation will be needed. Following this your surgery will proceed as normal. We will collect information from your biopsy results for the study and also your blood results from your normal follow up appointment after the surgery.

There are no extra hospital visits required for participation in this research project. There is no payment or vouchers for participation.

**What if I do not wish to take part?**

If you do not wish to take part there will be no change in your management. Your doctor will still be responsible for making the best possible decisions about your treatment.

**What happens if I change my mind during the study?**

You are free to withdraw at any time during the course of the study. This will not affect any treatment you might receive, now or in the future. If you decide to withdraw you just need to let us know. We would however like to keep any data which has already been collected.

January 2015  version 8.1
Whom should I contact for further information?

Mr Saba Balasubramanian, Senior Lecturer in Surgical Oncology, University of Sheffield (see below for details).

What if I wish to complain about the way in which this study has been conducted?

If you have any cause to complain about any aspect during the course of this study, the normal National Health Service complaints mechanisms are available to you. This process is not compromised in any way because you have taken part in a research study.

If you have any complaints or concerns please contact Mr Saba Balasubramanian (details provided below).

Alternatively, you can contact the Patient Services Team at Sheffield Teaching Hospitals on 0114 2712400 or via email at pst@sth.nhs.uk.

Or, you can use the normal University of Sheffield complaints procedure and contact the Research & Consultancy Unit, University of Sheffield, 2/4 Palmerston Road, Sheffield, S10 2TE.

Will my taking part in this study be confidential?

Yes. All information gathered about you during the course of the research will be kept strictly confidential. Data are handled only by the named researchers involved in the study. Personal data (name, address, etc) will not be transferred or transmitted outside the hospital. Your GP will however be informed of your participation in this study.

Am I entitled to compensation if something goes wrong?

In the event that something does go wrong and you are harmed during the research there are no special compensation arrangements other than those normally open to you in your treatment in the National Health Service. If the harm is due to someone’s negligence then you may have grounds for legal action for compensation against Sheffield Teaching Hospital NHS Foundation Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

January 2015 version 8.1
What will happen to the results from the study?

The study results will be presented at learned conferences of medical societies and published in peer-reviewed scientific journals.

Who is organising and funding this study?

The study is organised and carried out by the Department of Oncology at the University of Sheffield. The imaging technology is being provided by a commercial company (Fluoptics). The study is being supported (funded) jointly by Fluoptics and the University of Sheffield.

Contact name and numbers
Mr Saba Balasubramanian
Academic Unit of Surgical Oncology
E Floor, Royal Hallamshire Hospital
Sheffield, S10 2JF
Telephone: 0114 2261379
Email: s.p.balasubramanian@sheffield.ac.uk
A1.5 Participant information sheet (thyroid)

Sheffield Teaching Hospitals
NHS Foundation Trust

Mr S P Balasubramanian MS PhD FRCS, Senior Lecturer & Honorary Consultant Surgeon

Royal Hallamshire Hospital
Glossop Road
Sheffield
S10 2UF
Tel : 0114 271 1900
Fax : 0114 271 1901

Sarah Mason – Secretary to Mr Balasubramanian
0114 2712536 - Room F25
Fax: 0114 2713710

Participant information sheet (Thyroid)
Development of a clinical protocol to use near infra-red fluorescent imaging during thyroid and parathyroid surgery

We would like to invite you to take part in a research study. Before you decide to take part, it is important to understand why the research is being done and what is involved. This will help you make an informed decision. Please be reassured that your clinical care will not be affected by your decision.

One of our team will go through the information sheet with you and answer any questions you may have. This should take around 15 to 30 minutes. Please take time to decide and discuss it with others if you wish. Please ask us if there is anything that is not clear or if you would like more information.

Background to the study

The parathyroid glands are four tiny pieces of tissue. Each is about the size of a grain of rice. These glands are situated on either side of the thyroid gland in the neck. They produce a hormone called PTH (Parathyroid Hormone). PTH helps maintain blood calcium levels. Without parathyroid glands, the blood calcium level would fall. This can produce a range of unpleasant symptoms including tingling, pins and needles, fatigue, painful muscle cramping and if left untreated seizures. There is also potential for long term damage to the kidneys and bones.

During thyroid surgery, it is important to accurately identify parathyroid glands as they may sometimes be mistaken for other tissues in the neck. If the parathyroid glands are not identified during surgery, this may result in inadvertent damage to normal parathyroid glands.

This study aims to evaluate a new technique in the identification of both normal and abnormal parathyroid glands during parathyroid and thyroid surgery. If successful, the technique has potential to improve outcomes from these operations.
What do we do in this study?

As part of the study protocol, a dye (methylene blue) that tends to accumulate in parathyroid glands will be injected into your veins during the operation. This dye will be injected at a very low dose to start with. In some instances, a second dose may be injected. The total dose of dye used will be many times lower than the dose currently used in parathyroid surgery.

This dye has special (fluorescent) properties, which will be picked up by a recently developed fluorescent imaging system (Fluobeam®). The brightness of fluorescence is different for the different tissues/organs in the neck and will vary with time.

Recordings using Fluobeam® (and digital pictures) will be taken following the injections for a specified period of time during the operation. We hope to use the findings from the study to determine if the equipment will help the surgeon in identifying parathyroid tissue and help differentiate between normal and abnormal parathyroid tissue.

What are the possible benefits of taking part?

You will not directly benefit by taking part in this study. For future patients, if the technology aids in the detection of normal parathyroid glands which were not visible to the naked eye, the risk of inadvertent damage to these glands will be reduced. Also, if the technology appears to work in this phase of the study, it will be further tested in another clinical trial to see if the risk of complications following thyroid surgery and the rates of failure following parathyroid surgery can be lowered. This research therefore has potential to benefit patients undergoing thyroid or parathyroid surgery in the future.

What are the risks of taking part?

There is a small risk of allergic reactions from the dye and irritation or damage to the skin at the site of infusion. These problems are however very rare and can be treated if they arise.

The fluorescent imaging technology used during the operation to make recordings is unlikely to influence the nature of the operation or the outcomes.

There will however be some increase in the duration of the operation due to the need to make the observations. The maximum period of observation has been fixed at 10 minutes.

January 2015 version 8.1
Do I have to take part?

No. Taking part is completely voluntary. It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form. However, you are free to withdraw at any time. Whether you take part or not will not affect the standard of care you receive in any way at any time.

Obtaining informed consent for your planned operation is a separate process which will occur in parallel to the consent process for this research. This will be done by a member of the surgical team responsible for your care.

What will happen to me if I decide to take part?

You will have a discussion with a member of the research team. They will go through the details of the study and answer any questions you may have. You will then be asked to sign a consent form informing us that you agree to be involved. Three copies of this form will be made, of which one copy is for your records. You will have your surgery as scheduled. During the surgery you will have the dye injection, following which the surgery will pause for a few minutes whilst the fluorescent imaging device is used to observe the thyroid and parathyroid area. In some cases a second injection and a further period of observation will be needed. Following this your surgery will proceed as normal. We will collect information from your biopsy results for the study and also your blood results from your normal follow up appointment after the surgery.

There are no extra hospital visits required for participation in this research project. There is no payment or vouchers for participation.

What if I do not wish to take part?

If you do not wish to take part there will be no change in your management. Your doctor will still be responsible for making the best possible decisions about your treatment.

What happens if I change my mind during the study?

You are free to withdraw at any time during the course of the study. This will not affect any treatment you might receive, now or in the future. If you decide to withdraw you just need to let us know. We would however like to keep any data which has already been collected.

January 2015

version 8.1
Whom should I contact for further information?

Mr Saba Balasubramanian, Senior Lecturer in Surgical Oncology, University of Sheffield (see below for details).

What if I wish to complain about the way in which this study has been conducted?

If you have *any* cause to complain about *any* aspect during the course of this study, the normal National Health Service complaints mechanisms are available to you. This process is not compromised in any way because you have taken part in a research study.

If you have any complaints or concerns please contact Mr Saba Balasubramanian (details provided below).

Alternatively, you can contact the Patient Services Team at Sheffield Teaching Hospitals on 0114 2712400 or via email at pst@sth.nhs.uk.

Or, you can use the normal University of Sheffield complaints procedure and contact the Research & Consultancy Unit, University of Sheffield, 2/4 Palmerston Road, Sheffield, S10 2TE.

Am I entitled to compensation if something goes wrong?

In the event that something does go wrong and you are harmed during the research there are no special compensation arrangements other than those normally open to you in your treatment in the National Health Service. If the harm is due to someone’s negligence then you may have grounds for legal action for compensation against Sheffield Teaching Hospital NHS Foundation Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my taking part in this study be confidential?

Yes. All information gathered about you during the course of the research will be kept strictly confidential. Data are handled only by the named researchers involved in the study. Personal data (name, address, etc) will not be transferred or transmitted outside the hospital. Your GP will however be informed of your participation in this study.

January 2015  version 8.1
What will happen to the results from the study?

The study results will be presented at learned conferences of medical societies and published in peer-reviewed scientific journals.

Who is organising and funding this study?

The study is organised and carried out by the Department of Oncology at the University of Sheffield. The imaging technology is being provided by a commercial company (Fluoptics). The study is being supported (funded) jointly by Fluoptics and the University of Sheffield.

Contact name and numbers
Mr Saba Balasubramanian
Academic Unit of Surgical Oncology
E Floor, Royal Hallamshire Hospital
Sheffield, S10 2JF
Telephone: 0114 2261379
Email: s.p.balasubramanian@sheffield.ac.uk
A1.6 Consent form

CONSENT FORM - THYROID

Patient Identification Number: ___________________________ Date of birth: ___________________________
NHS/Hospital no.: ___________________________ Initials: ___________________________

Title of Project: Development of a clinical protocol to use intra-operative near infra-red fluorescent imaging in thyroid and parathyroid surgery

Principal Investigator: Mr Saba Balasubramanian

Please put your initials in each box if you agree with the corresponding statement.

1. I confirm that I have read and understand the patient information sheet for the above study, (version 8, thyroid surgery dated 02 Apr 15) and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. Data which has already been collected will be retained.

3. I understand that my clinical information and study data may be looked at by responsible individuals from the research staff, study sponsor (Sheffield Teaching Hospital NHS Foundation Trust), and from the regulatory authorities where it is relevant to my taking part in research; I give permission for these individuals to have access to my records

4. I understand that my medical information will be collected for use in this study and may be used to help develop new research. Data protection regulations will be observed and confidentiality will be maintained.

5. I understand that my GP will be informed of my participation in this study.

6. I agree to take part in the above study

Patient Signature ___________________________ Date ___________________________

Patient's name: ___________________________

Signature of Investigator ___________________________ Date ___________________________

taking informed consent

Investigator name: ___________________________

Original: Investigator Site File
1 Copy: Patient ☐
1 Copy: Hospital Notes ☐

STH17176 Consent Form (thyroid) version 8 date: 02 Apr 15

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A1.7 Data collection form

STH17176 - Intra-operative near infra-red fluorescent imaging in thyroid and parathyroid surgery

Patient ID [ ] [ ] Patient initials [ ] [ ]

Consent / Eligibility visit

Date of Informed Consent

Confirmation of Eligibility

Inclusion criteria

- undergoing a total thyroidectomy, hemithyroidectomy, targeted parathyroidectomy or bilateral neck exploration for PHPT

Exclusion criteria

- undergoing re-do procedure
- unable to understand spoken and written English
- unable to give adequate informed consent
- history of intolerance or sensitivity to MB
- known G6PD deficiency
- on serotonin reuptake inhibitors
- undergoing surgery for thyroglossal cyst
- undergoing thoracic exploration (alone or in combination with a neck exploration)
- pregnant or breast feeding
- eGFR of ≤ 59 ml/kg/1.73m²
  - Age less than 18 years at time of surgery

Gender: Male / Female DOB ______________

Date of pregnancy test ____/____/____ or NA Result: Positive / Negative

Date of latest eGFR ____/____/____ Result: _________ ml/kg/1.73m²

Group:

<table>
<thead>
<tr>
<th>Thyroidectomy</th>
<th>Hemithyroidectomy</th>
<th>Targeted Parathyroidectomy</th>
<th>Bilateral Neck Exploration</th>
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</thead>
</table>

Signature of investigator confirming eligibility ________________________________

Name ___________________________ Date ___________________________

STH17176 Case report form Version 1.2 16 Oct 2014
Date of surgery / IMP dosing ________________

Any adverse events since signing informed consent yes / no

If yes complete adverse events form

If SAE complete and fax/email SAE form

Record concomitant medications on con med form

Cohort Training / Test / Final

Indication for Surgery ____________________________________________

Procedure _____________________________________________________

Glands visible prior to methylthioninium chloride injection:

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<th>Gland visible?</th>
<th>Comments</th>
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<td></td>
<td></td>
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<tr>
<td>parathyroid tissue</td>
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</tbody>
</table>
Planned 1st dose strength of methylthioninium chloride ________ mg/kg

Reference source for first dose decision 

Weight ________ kg; Dose methylthioninium chloride: _____ mg/kg X _____ kg = _____ mg

Undiluted volume methylthioninium chloride 0.5%w/v to be infused (mg / 5) ________ ml

Drawn up by ____________________________  Checked by ____________________________

Given by ____________________________  Time of injection __________

Exposure Setting of Fluobeam ________ ms  Sequence Setting of Fluobeam ________ ms

Time of start of recording using Fluobeam __________

Side of neck recorded Left  Right

Fluobeam recording performed by ____________________________

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<thead>
<tr>
<th>Clearly identified by surgeon?</th>
<th>Any staining seen with naked eye?</th>
<th>If yes, time seen</th>
<th>Fluorescence seen with Fluobeam?</th>
<th>If yes, time seen</th>
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<tr>
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</tbody>
</table>

Completion of observation time (Fluobeam recording stop time) __________

Video Folder name (StudyId_bolusno._pic/videoono) ____________________________

Extravasation of MB at injection site? Yes / No

Intraoperative hypotension within 30 minutes of MB? Yes / No

Any other intraoperative event that the anaesthetist or other investigator thinks may be related to intravenous methylthioninium chloride? Yes / No

Any other intraoperative serious adverse event not thought to be related to methylthioninium chloride? Yes / No

If yes record details on adverse event form / serious adverse event form

Comments

Page completed by ____________________________  Date __________

STH17176 Case report form  Version 1.2  16 Oct 2014

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2nd dose of methyliothioninium chloride? Yes / No
If yes, strength to be administered ___ mg/kg
Weight _________ kg; Dose methyliothioninium chloride: ___ mg/kg X ___ kg = ___ mg
Undiluted volume methyliothioninium chloride 0.5% w/v to be infused (mg / 5) _________ ml
Drawn up by ___________________________ Checked by ___________________________
Given by _____________________________ Time of injection ___________________
Exposure Setting of Fluobeam ________ms Sequence Setting of Fluobeam ________ms
Time of start of recording using Fluobeam ______________
Side of neck recorded Left Right
Fluobeam recording performed by _____________________________

<table>
<thead>
<tr>
<th>Clearly identified by surgeon?</th>
<th>Any staining seen with naked eye?</th>
<th>If yes, time seen</th>
<th>Fluorescence seen with Fluobeam?</th>
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</tr>
</thead>
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<tr>
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</tr>
</tbody>
</table>

Completion of observation time (Fluobeam recording stop time) ______________

Video Folder name (Studyid_bolusno._pic/videon) ___________________________

Extravasation of MB at injection site? Yes / No
Intraoperative hypotension within 30 minutes of MB? Yes / No
Any other intraoperative event that the anaesthetist or other investigator thinks may be related to intravenous methyliothioninium chloride? Yes / No
Any other intraoperative serious adverse event not thought to be related to methyliothioninium chloride? Yes / No
If yes record details on adverse event form/ serious adverse event form
Comments
STH17176 - Intra-operative near infra-red fluorescent imaging in thyroid and parathyroid surgery

Patient ID [ ] [ ] Patient initials [ ]

Follow up Visit

Date of out-patient visit: ____________________________

Hypocalcaemic symptoms: yes / no

If yes record details ________________________________________

_____________________________________________________________________

Treatment for hypocalcaemia: yes / no

If yes record details on concomitant medication form

Any postoperative adverse events related to Methylthioninium Chloride: yes / no

If yes record details on adverse event form

Any postoperative serious adverse events yes / no

If yes record details on serious adverse event form

Adjusted Calcium ______ mmol/L Date of sample _______________________

Histology results

<table>
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<td>Supernumerary/suspected parathyroid tissue</td>
</tr>
</tbody>
</table>

Page completed by ____________________________ Date __________

STH17176 Case report form Version 1.2 16 Oct 2014

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Date fluobeam images reviewed: __________________________

Investigators reviewing images: __________________________

Disease group: Thyroid / Parathyroid

Study stage: Training / Test / Final

Methylthioninium dose 1: Dose strength _________ mg/kg

Any technical problems: __________________________

<table>
<thead>
<tr>
<th></th>
<th>Fluorescence (yes / no)</th>
<th>Time of onset of fluorescence</th>
<th>Time to peak fluorescence</th>
<th>Peak fluorescent intensity ratio (wrt muscle)</th>
<th>Fluorescence - Satisfactory / too much / too little</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td></td>
<td>NA</td>
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</table>

Page completed by: __________________________  Date: ____________

STH17176 Case report form  Version 1.2  16 Oct 2014  6
Methylthioninium dose 2  Dose strength __________ mg/kg  or  NA  if no 2nd dose

Any technical problems

<table>
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<tr>
<th></th>
<th>Fluorescence (yes / no)</th>
<th>Time of onset of fluorescence</th>
<th>Time to peak fluorescence</th>
<th>Peak fluorescent intensity ratio (wrt muscle)</th>
<th>Fluorescence - Satisfactory / too much / too little</th>
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<tr>
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</tr>
</tbody>
</table>

Stage complete?  Yes / No

If yes, next stage  Test / Final / Study complete

Decision of first dose for next patient in this group:

Maintain same first dose  /  Increase dose  /  Decrease dose

First dose strength for next patient in this group __________ mg/kg

Comments on dose decision

-------------------------

Page completed by ________________  Date ________________  16 Oct 2014
Principal Investigator Signature Statement

I have reviewed this DCF and confirm that to the best of my knowledge, it accurately reflects the study information obtained for this participant. All entries were made by either myself or by a person under my supervision who has signed the Delegation Log.

Principal Investigator's Signature ____________________________

Principal Investigator's Name ________________________________

Date of Signature __________________________________________
A1.8 Graphical representation of near infrared fluorescence from all patients

**Thyroid 0.1mg/kg**

Fluorescence Mean (Greyscale/ms)

Time (S)

**Thyroid 0.05mg/kg**

Fluorescence Mean (Greyscale/ms)

Time (S)
Thyroid 0.35mg/kg

Fluorescence Mean (Greyscale/ms)

Time (S)

0 30 60 90 120 150 180 210 240 270 300

- Parathyroid
- Thyroid
- Soft Tissue
- Drape

Thyroid 0.4mg/kg

Fluorescence Mean (Greyscale/ms)

Time (S)

0 30 60 90 120 150 180 210 240 270 300

- Parathyroid
- Thyroid
- Soft Tissue
- Drape
Parathyroid 0.4mg/kg

Fluorescence Mean (Greyscale/ms)

Time (S)

Thyroid 0.5mg/kg

Fluorescence Mean (Greyscale/ms)

Time (S)
A1.9 Raw data

Raw data used for analysis in Image J are available on an online repository (DOI: 10.15131/shef.data.19161170).
Appendix 2: Near infrared fluorescence in the pancreas

A2.1 Background

During the research period where MB NIRF technology was being investigated in the thyroid and parathyroids, a case arose of a pancreatic insulinoma in a symptomatic patient in whom localisation studies had not identified a mass. This was discussed at multidisciplinary team meetings and previous studies using this technology in the pancreas was discussed. It was agreed that we would trial MB NIRF in this patient as a one-off case study. Although this study does not fall into the remit of this thesis, it is an important case showing the versatility of MB NIRF in endocrine tissues.

A2.1.1 Pancreatic neuroendocrine tumours

Pancreatic endocrine cells arise from amine precursor uptake and decarboxylation (APUD) stem cells, which are pluripotent neuroendocrine cells found in the distal foregut and pancreas; in the latter, they cluster and form islets throughout the organ. Tumours that arise from these cells are called islet cell tumours or pancreatic neuroendocrine tumours (pNETs) as they have features of both neural and epithelial cells that secrete hormones and/or peptides (Kloppel, Perren et al. 2004). PNETs are much less common than pancreatic exocrine tumours with an incidence of <1 per 100,000 per year (Halfdanarson, Rabe et al. 2008, Elghazawy and Verbeke 2010). They are usually benign and if malignant have a better prognosis than exocrine tumours. Most pNETs are sporadic, but up to 30% of pNETs may be familial and usually linked to multiple endocrine neoplasia type 1 (MEN 1) or von Hippel-Lindau syndrome (Halfdanarson, Rubin et al. 2008).

PNETs may be non-functional or can secrete hormones such as insulin, gastrin, glucagon, vasoactive intestinal peptide (VIP) and somatostatin resulting in distinct clinical syndromes. Signs and symptoms of non-functional tumours are caused by growth and distant spread, most frequently to the liver and bone.

Diagnosis of pNETs is by baseline biochemical blood tests and systematic, multimodal imaging to localise the tumour for patients presenting with symptoms associated
with one of the functional syndromes. Surgery is the primary treatment for localised
tumours (Partelli, Maurizi et al. 2014, Partelli, Maurizi et al. 2014). Five-year survival
rates are around 60% in resectable cases (Bilimoria, Talamonti et al. 2008).
Treatment choices for non-resectable disease include somatostatin analogues,
biotherapy, targeted radionuclide therapy, locoregional treatments and
chemotherapy.

Pre-operative localisation of PNETs may be sometimes difficult, as these tumours are
often quite small; ultrasound and cross-sectional imaging sensitivity is only around
30% (Palazzo, Roseau et al. 1993, Anderson, Carpenter et al. 2000, Ardengh,
Rosenbaum et al. 2000). Intraoperative ultrasound, often assisted by the surgeon’s
ability to palpate the tumour, is perhaps the most helpful localisation technique if
preoperative imaging is not definitive. For multifocal and extensive disease, the
pancreas needs to be mobilised and assessed throughout its length to determine the
most appropriate extent of resection. It has been reported that primary surgical
resection has a 13% failure rate (Richards, Gauger et al. 2002); further surgery is
associated with a significantly increased morbidity including pancreatic or biliary
fistula, intra-abdominal abscess or peripancreatic fluid collection and pancreatitis.
Fistula rates following endocrine pancreatic surgery are higher than that following
adenocarcinoma resection (9.5-64.3% and 2-25% respectively) (Norton, Kivlen et al.
2003, Mulholland and Doherty 2011).

The aim of this study is to report on a case of insulinoma where the use of methylene
blue near infrared fluorescence technology was thought to be of value in the
localisation of a pancreatic neuroendocrine tumour.

**A2.1.2 Case discussion**

A 56-year-old Caucasian male presented feeling nauseated and dizzy with a facial
rash and uncontrolled right leg movement. Blood glucose reading was 1.2 mmol/L.
The patient was admitted for a 72 mixed meal test, which was stopped at 48 hours
after a blood glucose reading of 2.5 mmol/L. Biochemical investigations diagnosed an
insulinoma (Table A2.1, Table A2.2 and Table A2.3).
### Table A2.1  Biochemical Investigations

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<th>Investigation</th>
<th>Result</th>
<th>Reference Range</th>
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<td>4.0-5.9 mmol/L</td>
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<tr>
<td>Insulin</td>
<td>18</td>
<td>17.8 – 173 pmol/l</td>
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<tr>
<td>C-peptide</td>
<td>352</td>
<td>298 – 2350 pmol/l</td>
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<tr>
<td>IGF-1</td>
<td>17.1</td>
<td>97 – 292 μg/l</td>
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<tr>
<td>HbA1c</td>
<td>28</td>
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<td>Pro-insulin</td>
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<td>&lt;10 pmol/L</td>
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### Table A2.2  Mixed meal test results

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<th>Date</th>
<th>Time</th>
<th>Insulin (17.8 – 173 pmol/L)</th>
<th>C peptide (298 – 2350 pmol/L)</th>
<th>BMS venous mol/L</th>
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The lesion was difficult to localise with pre-operative imaging. The patient underwent magnetic resonance cholangiopancreatography (MRCP), MRI liver with contrast, CT, Octreotide scan and endoscopic ultrasound (EUS). Cross-sectional imaging did not reveal any abnormalities. EUS identified a small lesion near the head of the pancreas that was thought to be a lymph node. Histology from this lesion showed findings suggestive of a neuroendocrine tumour. There were nests and flat sheets of cuboidal cells with small, oval, salt and pepper patterned nuclei with conspicuous nucleoli and inconspicuous mitoses and granular and eosinophilic cytoplasm.
The patient underwent a period of continuous glucose monitoring (CGMS). Hypoglycaemic unawareness was a significant problem for this patient. CGMS showed widely varying glucose measurements (Figure A2.1).

The patient was treated with diazoxide and octreotide subcutaneous injections. However, this was poorly tolerated, and the patient had significant oedema and fixed facial flushing. On this basis, a decision was made for surgery. As pre-operative imaging had not clearly localised the lesion there was anticipated difficulty in localising the tumour intraoperatively and so intraoperative localising techniques were planned.

Previous case reports have described significant uptake of MB in the pancreas (Donald L Gordon 1974) and increased fluorescence from pancreatic tumours (Winer, Choi et al. 2010). Gordon et al published a case report of a 46-year-old female with an insulinoma who underwent open surgery. They identified a 1.6cm tumour in the pancreas and gave an infusion of methylene blue at 5mg/kg over 30 minutes. One hour after the end of the infusion the pancreas was inspected, and normal pancreatic tissue had stained light green/blue whereas the tumour was a deep reddish/blue. This study was one of the first studies looking at macroscopic uptake of methylene blue in the pancreas. It uses high doses of methylene blue, which can be associated with morbidity, and took 1.5 hours to have effect. In future cases the methylene blue infusion could be started prior to laparotomy but this could
lead to potential morbidity in patients who may be able to have their tumour
localised without aid at laparotomy. Winer et al published a study investigating
methylene blue NIRF in the pancreas in an animal study where intravenous boluses of
0.25-2 mg/kg methylene blue were injected into rats (n=15), pigs (n=4) and
insulinoma bearing transgenic mice (n=8). Whilst anaesthetised the animals
underwent laparotomy and the FLARE NIRF imaging system was used to inspect the
pancreas. They reported that doses of 1-2 mg/kg were required for adequate
fluorescence from the pancreas. They also found that the insulinoma had a greater
fluorescence than the surrounding pancreatic tissue, at 1.5 mg/kg the insulinoma-to-
pancreas ratio was 3.7. This study showed that reduced doses of methylene blue
could be used with NIRF imaging systems to localise insulinoma. The method of giving
an intravenous bolus is easily used intraoperatively. They noted that the fluorescence
had washed out of other abdominal organs in 2-5 minutes after the bolus but uptake
to the pancreas lasted up to 60 minutes. It was felt that methylene blue is not the
ideal fluorophore but that as it is widely accepted for use in medical procedures its
NIRF properties can be easily explored. This study only investigated insulinomas and
the authors commented that methylene blue fluorescence may not work in other
types of neuroendocrine tumours. There was good reason to postulate that the use
of MB and NIRF may help with tumour localisation. The use of MB at 1 mg/kg body
weight has a favourable risk profile, especially without concomitant use of selective
serotonin reuptake inhibitors (Patel, Chadwick et al. 2012). Given the potential
benefit, the use of this modality was discussed at the multidisciplinary meetings and a
further opinion was sought and obtained from the clinical governance lead. It was
concluded that any extra information to aid localisation of the tumour would be of
benefit and could potentially increase the chance of cure. An algorithm for the use of
NIR imaging was specified a priori to aid decision making during surgery (Figure
A2.2).
A2.2 Intraoperative Procedure

Under general anaesthesia, EUS was performed, and a small lesion was identified as before. A laparotomy was then performed, the lesser sac was opened and a full exploration (inspection and palpation) of the pancreas was done (Figure A2.3), but no clear lesion was identified. Intraoperative ultrasound was also used but was unsuccessful. It was decided that NIRF should be attempted.
The NIRF camera was covered in a sterile cover. With the ambient lighting dimmed, the laser and LEDs switched on and the camera in position to visualise the pancreas, an intravenous bolus of MB of 1mg/kg was administered by the anaesthetist and flushed with 5% Glucose. A transient fall in oxygen saturation was noted as expected within the first minute following injection. This recovered quickly and no adverse effects such as hypotension, extravasation or allergic reaction were noted.

The pancreas showed intense fluorescence. Reducing the exposure setting on the camera from 83ms to 40ms significantly improved the image quality. The pancreas gland was then systematically visualised with the camera starting with the body and tail. When the head of the pancreas was inspected, a very small lesion was seen fluorescing more brightly than the background pancreas. The surgeon was then able to localise this lesion within the pancreas. EUS was repeated to confirm that the lesion seen on NIRF corresponded with the lesion identified on EUS. The lesion was then enucleated.

Figure A2.3  Intra-operative surgical view

*Surgical field and surgical field with anatomical overlay. Location of tumour identified with arrow.*
The pancreas gland preferentially took up methylene blue. The pancreas showed fluorescence within 10 seconds of administration of the IV bolus. With the exposure set to 83ms, the pancreas gland showed increased uptake compared to background (subcutaneous fat) reading with a mean ratio of 3.9. The insulinoma showed increased fluorescence when compared to the surrounding pancreas with a ratio of 1.8. However, at this dose (1 mg/kg), the pancreas and insulinoma did not show differential staining to the naked eye.

A2.3 Patient Outcomes

The patient was discharged from hospital a week later following a routine recovery with no significant complications. In the immediate postoperative period, his symptoms had improved and a postoperative CGMS showed a normal trace (Figure A2.5).
Histopathology from the enucleated specimen reported a low grade neuroendocrine tumour with some weak insulin staining. However, the margins of this excised specimen were involved.

At one year, the patient largely remained asymptomatic with no requirement for medication. Repeated CGMS continued to show normal glycaemic control. However, the incomplete tumour resection was a cause for concern and the patient is undergoing further investigation.

**A2.4 Discussion**

As with endocrine tissues such as the thyroid and parathyroid glands, the pancreas preferentially takes up MB compared to other organs within the surgical field allowing visualisation with NIRF. The insulinoma appeared to have a greater affinity for the fluorophore compared to the background pancreas at a dose of 1mg/kg. This has the potential to allow even very small insulinomas to be differentiated from the normal gland.

Image resolution is likely to be improved by reducing the dose of methylene blue. The pancreas showed intense fluorescence at 1mg/kg, which degraded the quality of the images. Altering the exposure settings of the camera to reduce the “glare” from the pancreas improved the picture quality and allowed the tumour to be visualised against the background fluorescence from the pancreas.
Pre-operative localisation of insulinomas reduces the need for blind distal pancreatic resection, which is associated with increased re-operative morbidity, increased long-term diabetic related morbidity and premature deaths (Rostambeigi and Thompson 2009). Pre-operative localisation with non-invasive cross-sectional imaging in the form of CT or MRI has sensitivities of 33%-64% and 40%-90% respectively (Okabayashi, Shima et al. 2013). Invasive techniques including endoscopic ultrasound and arterial stimulation venous sampling are more accurate in localisation of insulinomas. EUS has a sensitivity of 86.6%-92.3% (Okabayashi, Shima et al. 2013). However, EUS success is dependent on operator experience and yields both false-positives and false negatives (Kann 2016). Insulinomas may also be isoechoic rendering EUS ineffective. Factors increasing negative results on EUS include low BMI, female gender and young age (Okabayashi, Shima et al. 2013). Sensitivity is increased for tumours located in the pancreatic head and decreased for those in the tail or extra pancreatic tumours (Sotoudehmanesh, Hedayat et al. 2007). The sensitivity of these techniques are all reduced when the tumour size is <1cm. Another invasive localisation technique, arterial stimulation venous sampling, localises insulinomas on the basis of their arterial supply and has reported sensitivity of 94%-100% (Okabayashi, Shima et al. 2013). This investigation is invasive and associated with complications including induced hypoglycaemia and pancreatitis (Mauro, Thomson et al.). Current British Association of Endocrine Surgeons (BAETs) guidelines suggest that CT, MRI, Octreotide Scanning and EUS should be used for pre-operative localisation. ASVS is only available at some centres. Patients should be treated with surgery, unless deemed unfit, even when pre-operative localisation has not identified the tumour and intra-operative techniques should be utilised to aid resection or enucleation.

Current intraoperative localisation is limited to manual palpation and intraoperative ultrasound. Sensitivity of these methods is increased in experienced hands and is reported as 75%-95% and 80-100% (Okabayashi, Shima et al. 2013). Fresh frozen section could confirm the presence of a neuroendocrine tumour. Blind resection is not recommended.
The question remains as to what should be done in the patients where both pre-operative and intra-operative localisation has failed to provide a definitive answer. Near infrared fluorescence may be useful as an alternative intraoperative investigation that can be performed quickly and with minimal risk to the patient. Other modalities of nuclear medicine scans are being developed including the $^{68}$Ga-DOTA-Extendin-4 PET/CT which has undergone pilot studies in Switzerland (Antwi, Fani et al. 2015) but are both expensive and not easily accessible to patients in the UK.

In conclusion, near infrared fluorescence technology may be useful in the intraoperative localisation of pancreatic insulinomas. Further investigation is required to optimise the dose of methylene blue and validation of our findings in a cohort is required.
Appendix 3: Appendices to Chapter 3

A3.1 Ethical opinion

NHS
Health Research Authority
NRES Committee Yorkshire & The Humber - Sheffield
Jarrow Business Centre
Viking Business Park
Rolling Mill Road
Jarrow
Tyne and Wear
NE32 3DT

Telephone: 0191 4283564

16 July 2015

Mr Sabapathy Balasubramanian
Senior Clinical Lecturer and Honorary Consultant Surgeon
University of Sheffield
EU35, E Floor
Royal Hallamshire Hospital
Beech Hill Road
S10 2RX

Dear Mr Balasubramanian

Study title: Phase 1 study of Electrical Impedance Spectroscopy in Thyroid and Parathyroid Surgery
REC reference: 15/YH/0293
Protocol number: n/a
IRAS project ID: 168701

The Research Ethics Committee reviewed the above application at the meeting held on 06 July 2015. Thank you for attending with Miss Sarah Hillary, Student Investigator, to discuss the application.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager Miss Kathryn Murray, nrescommittee.yorkandhumber-sheffield@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.
1. Submit a revised participant information sheet to address the following issue:

A Research Ethics Committee established by the Health Research Authority
a. Within the section entitled ‘What do we do in the study?’ amend the second sentence to read ‘This probe may allow the surgeon to identify parathyroid gland tissue during surgery.’

2. Submit a revised consent form to address the following issue:
   a. Revise consent point four to clearly state that the medical photographs which would be taken would be of the area on which the participant is being operated.

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which can be made available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission (“R&D approval”) should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

 Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at [http://www.rdforum.nhs.uk](http://www.rdforum.nhs.uk).

Where a NHS organisation’s role in the study is limited to identifying and referring potential participants to research sites (“participant identification centre”), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from NRES. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS Sites

A Research Ethics Committee established by the Health Research Authority
The favourable opinion applies to all NHS sites taking part in the study taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see “Conditions of the favourable opinion” below).

Non NHS sites

The Committee has not yet completed any site-specific assessment(s) (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. I will write to you again as soon as an SSA application(s) has been reviewed. In the meantime no study procedures should be initiated at non-NHS sites.

Summary of discussion at the meeting

Social or scientific value; scientific design and conduct of the study

The Committee acknowledged the link between this proposed study and previous research proposals and requested an update on the progress of the existing studies.

You explained that the initial application which was made about 2.5 years ago was for Phase IA study on 10 participants which has now been completed and found interesting results. You advised that there was now a Phase IB underway which was approved through a Manchester REC. You explained the proposed study would complement the existing research and were not competing technologies.

You advised that the Phase IA study had developed a test device utilising the flourance properties of methylene blue which was now being used in the Phase IB study along with different doses of Methylene Blue in order to understand what the correct dosage was. It was confirmed that this study would be ongoing in parallel with the proposed research and you confirmed that there were already 11 out of the required 40/50 participants recruited to this trial, with the recruitment expected to be completed by October 2015, when the proposed trial recruitment would begin.

Clarification was requested around how a Clinician would find the parathyroid glands in a patient.

Miss Hillary confirmed that at the present time this was down to the surgeon’s experience and knowledge of anatomy. She added that there were four places to look for the parathyroid glands and it would usually be found.

The Committee queried how often surgeons misidentified the parathyroid.

You explained that within your Trust there was a 25% ‘damage rate’ to these glands during surgery, which was similar to the national average which was between 25-30%. You added that there was an issue with identifying these glands in surgery which needed to be addressed as damage to them can lead to hypocalcaemia. You explained that the parathyroid glands can often be mistaken for lymph nodes or brown fat tissue in surgical practice, which is why further research is required around their identification.

The Committee requested further information around how this device could be used to differentiate between the thyroid and parathyroid tissue. Miss Hillary explained that the device is currently being used in cancer treatment to identify cervical cancer cells. She explained that it was thought that the technology could be used in surgical practice to identify the parathyroid from the thyroid tissue as there should be different waves travelling through the different cells. She explained that when the surgeon identified the parathyroid tissue, a reading would be taken on the device at this point, before

A Research Ethics Committee established by the Health Research Authority
the blood supply to the area is changed. She confirmed that patient having both thyroid and parathyroid surgery would be included in the study as the parathyroid tissue would need to be identified in all instances.

Members queried how the device reading would impact on the surgery which was taking place.

*Miss Hillary explained that the device readings would not be seen at the time of surgery so this would not have an impact on the surgical procedures the surgeon would not know the reading until after the procedure had been completed. She further explained that the device was not being used in this project to assist with locating the parathyroid glands, adding that the purpose was to use the instrument to give a reading in vivo of the frequency wave through parathyroid cells.*

*She added that this was why the project had been separated into two cohorts to enable good frequency readings to be gathered through a variety of cells including the thyroid, muscle, tissue, fats as well as parathyroid when positively identified. If the planned surgery was to remove the parathyroid glands, the readings would be taken prior to its removal.*

The Committee requested further clarity around why it was believed that the electrical impedance would differ between the thyroid and parathyroid cells.

*Miss Hillary explained that this was due to the cell structure, as the parathyroid cells were tightly packed whereas the thyroid cells were less compact, which would give different electrical impedance.*

The Committee asked if the investigations could be carried out on animal tissue.

*Miss Hillary explained that her predecessor had carried out investigations on rabbits, as they were most comparable with human tissue, and found that there was a difference, with parathyroid and lymphnode tissue giving a lower frequency reading. She explained that this could not be extrapolated to humans as the genetic makeup was not exactly the same.*

**Recruitment arrangements and access to health information, and fair participant selection**

The Committee queried whether there had been any issues with recruitment to the previous trials.

*Miss Hillary explained that for the initial Phase IA trial 13 patient were approached in order to get the 10 participants required. She explained that recruitment had been more challenging in the Phase IB trial due to the additional time which was quoted in the participant information sheet for the study procedures. She added that after a number of procedures had been carried out, it was found that the trial procedures took a much shorter time than was originally quoted, so a substantial amendment was submitted to amend the participant documentation to show more accurate timings and no further issues were encountered with recruitment.*

**Favourable risk benefit ratio; anticipated benefit/risks for research participants (present and future)**

Members queried how long taking the device readings would add to the overall surgical procedure and whether this had any implications for the patients anaesthetic.

*You explained that it would take an additional 10 minutes as a maximum to take a variety of readings on the device which would not be detrimental to patient care.*
Informed consent process and the adequacy and completeness of participant information

The Committee advised that there were some minor issues with the participant information sheet and consent form which would be raised in correspondence.

Other general comments

The Committee requested further information around the funding arrangements for the study.

You explained that Silico were managing the device and its development, adding that it had been through various clinical trials around cervical cancer and were now looking at oral cancers. You explained that the research team approached Silico about the project and explained that they had agreed to provide the device, consumables and expert advice for the study. Some additional funding was being provided for the University of Sheffield. You explained that there was no additional external funding at this point as the research team wanted to carry out this initial pilot study, before moving onto a larger trial.

The Chair thanked the applicants for their attendance and they left the meeting.

Other ethical issues were raised and resolved in preliminary discussion before your attendance at the meeting.

Approved documents

The documents reviewed and approved at the meeting were:

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
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<tr>
<td>Instructions for use of medical device [ZedScan Instructions For Use]</td>
<td>v1.2.1</td>
<td>01 October 2013</td>
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<tr>
<td>Participant information sheet (PIIS) [STH18736 EIS Participant Information Sheet, v1.0 21May2015]</td>
<td>v1.0</td>
<td>21 May 2015</td>
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<tr>
<td>REC Application Form [REC_Form_05062015]</td>
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<td>05 June 2015</td>
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<tr>
<td>Summary CV for Chief Investigator (CI) [S Balasubramanian CV]</td>
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<td></td>
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<tr>
<td>Summary CV for student [S Hillary CV]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summary CV for supervisor (student research) [N Brown CV]</td>
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</table>

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document “After ethical review – guidance for researchers” gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

A Research Ethics Committee established by the Health Research Authority
• Notifying substantial amendments
• Adding new sites and investigators
• Notification of serious breaches of the protocol
• Progress and safety reports
• Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

15/YH/0293 Please quote this number on all correspondence

With the Committee’s best wishes for the success of this project.

Yours sincerely

pp.
Professor Basil Sharrack
Chair

E-mail: nrescommittee.yorkandhumber-sheffield@nhs.net

Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments

“After ethical review – guidance for researchers” [SL-AR2 for other studies]

Copy to: Jemima Clarke, Sheffield Teaching Hospitals NHS Foundation Trust
### NRES Committee Yorkshire & The Humber - Sheffield

#### Attendance at Committee meeting on 06 July 2015

#### Committee Members:

<table>
<thead>
<tr>
<th>Name</th>
<th>Profession</th>
<th>Present</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr Paul Bacon</td>
<td>Lead Clinical Scientist in Audiological Science, Medical Physics</td>
<td>Yes</td>
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</tr>
<tr>
<td>Mrs Jacqui Gath</td>
<td>Retired Senior Systems Analyst</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Professor Frank Jones</td>
<td>Retired Professor of Polymers and Composites</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Mr Pete Laud</td>
<td>Statistical Consultant</td>
<td>No</td>
<td></td>
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<tr>
<td>Mr Richard Lindley</td>
<td>Consultant Paediatric Surgeon</td>
<td>No</td>
<td></td>
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<tr>
<td>Dr Marie Marron</td>
<td>BHF Research Fellow</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Mrs Jennifer Martin</td>
<td>Retired Pharmacist</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Dr Amaka Offiah</td>
<td>Reader in Paediatric Musculoskeletal Imaging</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Professor Basil Sharrack (Chair)</td>
<td>Consultant Neurologist</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Dr Soon Song</td>
<td>Consultant Diabetologist</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Mrs Yvonne Stephenson</td>
<td>Lead Technician in the Department of Infection and Immunity</td>
<td>Yes</td>
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<tr>
<td>Mrs Helen Teasdale</td>
<td>University Executive Assistance</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Dr Nana Theodorou</td>
<td>Specialist Orthoptist</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Dr Steven Thomas</td>
<td>Consultant Vascular and Cardiac Radiologist</td>
<td>Yes</td>
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#### Also in attendance:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position (or reason for attending)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miss Kathryn Murray</td>
<td>REC Manager</td>
</tr>
<tr>
<td>Mr Sabapathy Balasubramanian</td>
<td>Chief Investigator</td>
</tr>
<tr>
<td>Miss Sarah Hillary</td>
<td>Student Investigator</td>
</tr>
</tbody>
</table>
A3.2 Study protocol

TITLE: Development of a clinical protocol to use Electrical Impedance Spectroscopy in Thyroid and Parathyroid Surgery

Chief Investigator
Mr Saba Balasubramanian,
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Email: s.p.balasubramanian@sheffield.ac.uk

Principal Investigator
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University of Sheffield
Email: sarah.hillary@doctors.org.uk

Sub-Investigators
Dr Peter Highfield, Technical Director, Zilico Ltd
Prof Nicky Brown, Department of Oncology, University of Sheffield
Prof Brian Brown, Emeritus Professor, University of Sheffield

Study sponsor
Sheffield Teaching Hospital NHS Foundation Trust

STH project reference number
STH18736

Protocol number, version and date
Protocol_DRAFTv1.1 – written by SH September 2014
Protocol DRAFT v1.1_spb – amended SPB 11th Nov 2014
Protocol DRAFT v2 – amended SH 14th Nov 2014
Protocol DRAFT v3 – amended SH 26th March 2015
Protocol DRAFT v4 - amended by SH and JC 07th May 2015
Protocol DRAFT v5 – amended by SH 08th May 2015
1 Research Questions

Hypothesis: Intra-operative use of electrical impedance spectroscopy technology will facilitate early and accurate identification of parathyroid glands and thereby improve outcomes following thyroid and parathyroid surgery.

2 Abstract

Thyroid and parathyroid operations are common endocrine procedures; around 13,000 are performed each year in the UK for both malignant and benign diseases (Vanderpump 2011). The thyroid gland is located in the central neck compartment and there are usually four parathyroid glands surrounding the thyroid, with two either side. However, the location and number of parathyroid glands may vary greatly (Akerstrom, Malmaeus et al. 1984, Lappas, Noussios et al. 2012). Identification of the parathyroid glands is a crucial step in both thyroid and parathyroid surgery and is critical to the success of the operation. In thyroidectomies, inadvertent injury, or even excision (Pattou, Combemale et al. 1998, Sakorafas, Stafyla et al. 2005), of the parathyroid glands may cause hypocalcaemia (low calcium levels) (Bergenfelz, Jansson et al. 2008, Edafe, Antakia et al. 2014), which can be either temporary or, in some cases, permanent and requires treatment with supplements (Khan, Waguespack et al. 2011). Long-term treatment is associated with significant morbidity (Mitchell, Regan et al. 2012). In parathyroid surgery, the glands must be identified to decide which gland(s) are abnormal and should be excised. Prompt identification of abnormal (enlarged) glands reduces the risk of injury to normal glands and therefore increases the chances of normal calcium levels postoperatively (Antakia, Edafe et al. 2014).

Currently there are a number of preoperative or intraoperative methods of facilitating identification of a parathyroid adenoma including ultrasound, MIBI scan, frozen section, gamma probe, intra-operative PTH assay and Methylthionium Chloride (MB) (Chien and Jacene 2010). However, none of these methods are useful in the identification or localisation of the normal parathyroid gland. Electrical impedance spectroscopy (EIS) uses the difference in resistance (electrical impedance or EI) properties of tissues to differentiate between different tissue types.
(Dean, Ramanathan et al. 2008). We have demonstrated in animal models that the differences in EIS at a range of frequencies enabled the clear differentiation of one tissue type from another in vivo (Antakia, Gayet et al. 2014) using a handheld device called the Zilico APX100. EIS has not been studied in the human neck previously. This study will aim to study the impedance patterns of different soft tissue structures in the human neck to determine if EIS can help in differentiation of human thyroid, parathyroid, adipose tissue and lymph nodes using a modified version of the APX100 called ZedScanTM.

3 Aims of study

The aims of this study are:

1. To determine EIS spectra of normal and abnormal thyroid, parathyroid, lymph nodes, fatty tissue and skeletal muscle

2. To compare the EIS patterns of the parathyroid with the other soft tissue structures

3. To investigate changes in EIS before and immediately after excision of soft tissue structures in thyroid and parathyroid surgery

4. To develop a protocol for the use of EIS in the accurate differentiation of parathyroid glands from other structures including thyroid nodules, adipose tissue and lymph nodes.

4 Background

Normal parathyroid glands (PGs) are at risk of inadvertent damage or devascularisation during both thyroidectomy and parathyroidectomy. In thyroidectomies preservation of the PGs is essential to reduce the risk of transient or permanent hypoparathyroidism with resulting hypocalcaemia (Sousa Ade, Salles et al. 2012, Edafe, Antakia et al. 2014) due to devascularisation or obstruction of venous drainage (Youngwirth, Benavidez et al. 2010). PGs that have been visualised can be preserved or, if the blood supply has been compromised, re-implanted at the time of surgery (Lo 2002). It is not uncommon for a PG to be inadvertently excised
with the thyroid tissue (Pisanu, Cois et al. 2003, Sakorafas, Stafyla et al. 2005, Page and Strunski 2007, Sorgato, Pennelli et al. 2009). During neck exploration for primary (PHPT) or renal hyperparathyroidism, both normal and abnormal glands often need to be identified prior to excision of abnormal glands (Kebebew and Clark 1998); therefore accurate identification is crucial to the success of the operation. This is often straightforward in patients with single gland disease; however, this can be challenging in patients with multi gland disease (accounting for 10-15% of patients with PHPT and all patients with renal hyperparathyroidism (Pitt, Sippel et al. 2009)). This is reflected by the increased incidence of persistent/recurrent disease in this subset of patients. There are usually four PGs (two on either side of the thyroid gland); each around 3-5mm in size. They can vary in number and location (Akerstrom, Malmaeus et al. 1984, Lappas, Noussios et al. 2012), and can often be difficult to distinguish from other common structures in the central neck compartment such as thyroid nodules, adipose tissue or lymph nodes (Akerstrom, Malmaeus et al. 1984, Lappas, Noussios et al. 2012).

Postoperative acute hypocalcaemia can manifest itself with a number of symptoms including circumoral paraesthesia, tetany, carpopedal spasm, laryngospasm, echocardiogram (ECG) changes and even cardiac arrest in severe cases (Khan, Waguespack et al. 2011). Management of hypocalcaemia requires replacement therapy with calcium and vitamin D supplements and close biochemical monitoring (Pattou, Combemale et al. 1998, Schaffler 2010, Khan, Waguespack et al. 2011, Fong and Khan 2012). Patients with permanent hypocalcaemia must have life-long supplements and can develop significant morbidity including renal stones, nephrocalcinosis and soft tissue calcification (Sitges-Serra, Ruiz et al. 2010).

There are currently a number of preoperative or intraoperative methods of facilitating identification of a parathyroid adenoma including ultrasound, MIBI scan, frozen section, gamma probe, intra-operative PTH assay and Methylthionium Chloride (MB) (Kebebew, Arici et al. 2001, Haciyanli, Lal et al. 2003, Sebag, Shen et al. 2003, Uruno and Kebebew 2006, Harrison and Triponez 2009, Chien and Jacene 2010, Patel, Chadwick et al. 2012). However, none of these methods are useful in the identification or localisation of the normal parathyroid gland.
Electrical impedance (EI) is the measure of resistance that a circuit poses to an alternating current when voltage is applied. When tissue is included as part of the circuit, the difference in EI can be used to differentiate tissue types (Dean, Ramanathan et al. 2008). Differences in cellular structure and constituents including lipid content and nuclear size have an effect on EI. The difference in EI also varies across a range of frequencies. As the structure of the thyroid and parathyroid glands are quite different it has been shown in animal models that at low frequencies, the EI was significantly higher in thyroid tissue than that of PGs (Antakia, Gayet et al. 2014). The thyroid gland contains follicles that store thyroglobulin which are lined with a single layer of epithelial cells. Calcitonin secreting parafollicular cells interposed between follicles. PGs are made of chief and oxyphil cells, which are densely packed. EI is also dependent on ion mobility, which is affected by temperature-induced fluid and electrolyte shifts within the tissues. It has been shown that the EI of a tissue rises by 2% with each unit drop in °C (Edd, Horowitz et al. 2005, Liu and Zhang 2014). Electrical impedance spectroscopy (EIS) has been used previously in humans in detecting malignancy. In prostate tissue it was demonstrated that there were different EI patterns in normal and malignant tissue; it is thought that this has potential to improve the accuracy of prostate cancer diagnosis (Halter, Schned et al. 2008, Mishra, Bouayad et al. 2012, Liu and Zhang 2014). EIS has been studied in diagnosis of high-grade cervical intraepithelial neoplasia (HG-CIN) during colposcopy and increased the positive predictive value (PPV) of diagnosis of HG-CIN during colposcopy from 53.5% to 67% (Tidy, Brown et al. 2013). EIS has also been combined with mammography in breast cancer screening. It increased the rate of detection of suspicious and malignant features from 67% to 83% when compared to the American College of Radiology (ACR) BIRADS numerical ranking system of mammograms (Kerner, Paulsen et al. 2002). General Practitioners have also compared EIS to traditional visual screening techniques in the diagnosis of malignant melanoma; EIS was shown to have higher sensitivity and specificity (Aberg, Nicander et al. 2004, Aberg, Birgersson et al. 2011, Malvehy, Hauschild et al. 2014).

If human thyroid and parathyroid tissues have a discernible, consistent and reproducible difference in EI, this has potential to be used intra-operatively for
accurate identification of parathyroid glands. This would lead to potential improvements in surgical outcomes in both thyroid and parathyroid operations.

Zilico have developed the ZedScan™ system which consists of a handheld device and a docking station which downloads data to a laptop computer. A disposable, single use sensor covers the tip of the hand-held unit. The tip of the sensor is 5.5mm in diameter and has four silver/silver chloride electrodes, which are placed in contact with the tissue. The diameter and spacing of the four electrodes determine the flow of current across the tissue. The current (<12 μA p-p) is passed between an adjacent pair of electrodes and the impedance is measured between the other pair. Each measurement is performed at 14 frequencies (ranging from 76 to 625000 Hz) in about 20 milliseconds. The data from the measurements is then downloaded to a laptop and transformed into a measure of mean EI and standard deviation (SD) at each frequency. We have demonstrated in animal models that at a range of low frequencies there is a significant difference in the EI of thyroid, parathyroid and muscle. The Phase I study will include patients undergoing thyroid and parathyroid surgery and will evaluate the EI of thyroid, parathyroid, fat, lymph nodes and strap muscles at a range of frequencies. This will enable the development of a protocol for the use of the ZedScan™ in routine thyroid and parathyroid surgery.

To perform EI measurements with ZedScan™ in human surgery, a series of experiments have been conducted by Synergy Health to determine the appropriate sterilisation procedure for the sensor tips both to ensure patient safety and effective measurement of impedance. In stage one, boxes of the sensor tips were subjected to different levels of gamma irradiation (15-45kGy) and samples from each box were assessed for physical appearance, mechanical properties and electrical performance. These tests demonstrated that the tips could survive the high radiation doses. Stage two determined the minimum dose required to sterilise the sensors as 25kGy. Sensors have been irradiated and then tested for bioburden according to the relevant standard. Stage three (to be done close to the beginning of the trial) will prepare the final batch of sensor tips ready for use. This is done on the basis of the appropriate radiation dose (between 24-45kGy) based on results of experiments in
stages one and two. A small number of these sensors will be tested again to ensure function and sterility.

5 Plan of investigation

Methodology

Patients undergoing thyroid and/or parathyroid surgery will be included. Both these groups are being included as identification of thyroid and/or PGs are a routine part of these operations and will provide EI measurements from both normal and abnormal parathyroid and thyroid tissues. There is potential for EIS to be useful in all of the above procedures.

At surgery (usually carried out under general anaesthesia), the thyroid and/or parathyroid gland(s) in the central compartment of the neck are exposed and mobilised prior to excision. During the procedure and prior to devascularisation and excision of the glands, the handheld ZedScan™ will be used to take in vivo measurements of thyroid, parathyroid, adipose tissue, lymph node and muscle. Each measurement is automatically performed at 14 different frequencies (ranging from 76 to 625000 Hz) in around 20 milliseconds. The measurement will be repeated soon after excision of the appropriate structures to enable ex vivo readings. The temperature of the patient at the time of the in vivo measurements will be recorded by the anaesthetist and the temperature of the specimen at the time of the ex vivo measurements will also be recorded. Care will be taken to apply the same amount of pressure to each tissue type. Readings will be taken in a specific order depending on the operation. This is illustrated in the following table (Table 1):
Any extra readings will be clearly documented in the case report forms (CRF). A photograph of the operating field may be taken for reference when analysing the measurements. After taking the measurements, the data is downloaded to a laptop computer and transformed into a measure of mean EI and SD at each frequency.
Summary of study design

This is an interventional study without a control arm.

Setting for the project

This study must take place in theatre intra-operatively whilst the patient is under general anaesthetic.

Participants

All adult patients (18 years and over) undergoing thyroid and/or parathyroid surgery in Sheffield Teaching Hospitals NHS Foundation Trust (STH) will be eligible for inclusion in this study.

Exclusion Criteria:

Patients undergoing thyroglossal cyst surgery, re-do procedures only involving resection of lymph nodes

Patients unable to understand spoken and written English

Patients unable to give adequate informed consent

Patients with a positive pre-operative pregnancy test

Sample size

Accurate sample size calculations are not possible as this is a pilot study and there is no previous data of electrical impedance on human thyroid and parathyroid tissue. An animal study of nine rabbits demonstrated significant differences between thyroid and parathyroid tissue and between in vivo and ex vivo specimens (Figure 1). In the human study, the measurements will be taken from both normal glands and abnormal glands; the latter may be involved in a number of different benign and malignant pathologies. As EI may differ based on pathology, it would be reasonable to include a number of patients for each of the different thyroid and parathyroid pathologies commonly encountered (colloid goitre, thyroid cancer, Graves' disease,
parathyroid adenomas and hyperplasia). At an approximate estimate of 10 patients for each of the five pathologies described, we aim to recruit around 50 patients with an approximately even distribution between those with thyroid and parathyroid disease. On this basis, around 25 patients in each group (thyroid and parathyroid surgery) will be recruited in this study.

**Figure 1**

[Graph showing median in vivo impedance of thyroid, parathyroid, and muscle across different frequencies]

**Recruitment**

Patients undergoing thyroid and parathyroid surgery for both malignant and benign disease in STH are managed in the Endocrine Surgical Unit of the Directorate of General Surgery. Patients suitable for inclusion in the study will be identified by clinicians and approached by the consultant, registrar or clinical research fellow at
their visit to hospital. This may either be at the outpatient clinic when they are listed for surgery or the pre-assessment unit in Sheffield Teaching Hospitals, Sheffield. Information about the study will be presented and those expressing an interest in the study will be provided with (or sent by post) a Participant Information Sheet and have the study explained in detail. Patients will not be required to indicate their willingness to participate on the same day. Any further questions or clarifications will be addressed either by telephone conversation or during subsequent visits to hospital. These patients will then have a second consultation on a different day from a member of the same team to answer any questions and to gain written consent. This will usually either be at the pre-assessment unit or the theatre admissions unit on the day of surgery. All patients willing to take part in the study will be requested to sign the consent form and the research team will ensure that the patients are fully informed of the implications of taking part in the study.
Recruitment Flow chart

Patient placed on waiting list for surgery

Patient informed about study

Patient interested?

No further action

Yes

Add to screening

First meeting (outpatient or pre-assessment clinic): Give information about the study and participant information sheet

Opportunity to contact research team

Review of notes for eligibility

Not eligible

Withdraw from study

Second meeting (pre-assessment clinic or day of surgery): Answer any questions, take informed, written consent

Withdraw from study

Eligible

South

Consent

Given

Declined

Withdraw from study

Add to enrolment log

Participate

Follow up appointment

Completed patient
Outcome measures

The primary outcome will be the generation of electrical impedance spectral curves (EI across the range of frequencies) for thyroid, parathyroid, muscle, lymph nodes and adipose tissues.

Statistical analysis

The EI spectra for each of the individual organs will be presented in graphical and text form. The EI spectra of the various organs will be compared graphically, and exploratory analyses will be performed with the aim of determining appropriate cut-offs/threshold levels of EI that will help differentiate parathyroid EI from all the other soft tissue structures. For individual organs, the in vivo and ex vivo EI will be compared using parametric or non-parametric tests, as appropriate. Further exploratory analyses will also be performed to study differences in the EI spectra of thyroid and parathyroid tissue across different thyroid and parathyroid pathologies.

Intervention

In patients who have provided informed, written consent, the operation will be performed as clinically indicated. During the procedure as certain soft tissues are exposed, readings will be taken using the ZedScan™ probe. Tissue that is excised as part of the planned operation will also have readings taken from it prior to dispatching it to the laboratory for histological analysis.

The subsequent operation and post-operative care will be routine. For the purposes of the study, the histology results will be accessed to compare histological confirmation of the tissue type to that documented by clinical judgement and any pathology identified within that tissue. The participant will not have to undergo any extra tests or attend extra hospital appointments for the purposes of the study.

Safety assessments

The ZedScan™ device is CE marked for use in colposcopy and is safe to use in humans.
To perform EI measurements with ZedScan™ in human surgery, a series of experiments have been conducted by Synergy Health to determine the appropriate sterilisation procedure for the sensor tips both to ensure patient safety and effective measurement of impedance. Two stages of these experiments have been completed and the third will be done close to the commencement of the study.

Stage one – Sensor tips were tested for appearance, mechanical properties and electrical performance following gamma radiation (15-45kGy). The sensors can survive the highest dose of radiation. This has been tested both by Zilico and Clinical Engineering at STH.

Stage two – Sensor tips were tested for bioburden following radiation. This determined the minimum dose required to sterilise the sensors as 25kGy.

Stage three – This stage will produce the final batch of sensors for use. A sample of sensors will be tested again to ensure function and sterility prior to use.

**Pregnancy**

Patients with a positive pre-operative pregnancy test will be excluded.

**Subject withdrawal, breaking the blind and trial stopping/discontinuation rules**

Any patients wishing to withdraw from the study at any stage following initial inclusion will be free to do so. Any data already collected will be retained.

**Instruments**

The ZedScan™ is a technology developed by Zilico, a commercial company currently based in Manchester, UK. The product is CE marked and does not pose any direct risk to the patient. It currently holds a CE mark for use in colposcopy and is used in the Colposcopy department at Sheffield Teaching Hospitals. The investigators are familiar with the workings of the device. The ZedScan™ currently costs around £3000. This study does not require any additional infrastructure or equipment for its application.
Quality control & assurance

The results of the study will be audited by the Research and Development Department at Sheffield Teaching Hospitals.

Project plan

It is feasible that this study can be completed within 9-12 months with the current numbers of patients undergoing endocrine surgery at Sheffield Teaching Hospitals.

6 Statistical opinion

This is an exploratory, pilot study of EI in soft tissue structures of the human neck. In the absence of preliminary data, precise sample size calculations are not possible. However, estimates of required sample size and intended analyses have been described.

7 Project management

Clinicians and researchers will work together to identify, screen and consent patients in a timely fashion. Monthly meetings will be held between the Chief investigator and Primary Investigators to review progress and data collected. Input from Zilico may be sought to help with analysis of the spectra produced. The chief investigator has overall responsibility, but the primary investigator is responsible for the day-to-day running of the study.

8 Expertise of the researcher and associated team

The surgeons in Sheffield perform around 250 thyroid and/or parathyroid surgeries a year at Sheffield Teaching Hospitals and are well placed to recruit the required number of patients for this study. Sheffield Teaching Hospitals has a good relationship with Zilico as the technology was developed here. The chief investigator has already used an earlier model to conduct a small animal study. Support from clinic and theatre staff has been sought to aid co-operation in this study.
9 Ethical issues

The study will only begin after being approved by the Research Ethics Committee (REC) and NHS Trust. The study will be performed in accordance with recommendations guiding physicians in biomedical research involving human subjects (Declaration of Helsinki). Informed written consent will be obtained from all patients prior to entry into the study. The right of the patient to refuse participation without giving reason will be respected and patients will remain free to withdraw their consent for the storage of data in the study at any time without giving reasons and without prejudicing future treatment. Any amendment to the protocol will only be made with the approval of the chief investigator and Sponsor and changes to the study will be subject to review by the REC. The trial will be registered with a trials registry (www.clinicaltrials.gov).

The members of the research team will have attended the good clinical practice (GCP) training by the time of the start of the study.

The device has a single-use, disposable tip that will be sterilised for the purpose of the study. The handpiece will be covered by a sterile plastic cover, which is currently used in theatre to cover similar probes. The use of ZedScan™ therefore does not pose an infection risk to patients. The device is portable and may be transported easily to the operating theatre. The additional time required for the use of this device during surgery is minimal as no further dissection than routinely performed is required and the measurements are taken in a very short period of time (few seconds to minutes). Only tissue excised as part of the operation will have ex vivo measurements taken.

Confidentiality

The study team will be responsible for recording findings and collecting data in the trial. Summary data and the results of analyses from this study will be used to write a report for presentation, publication and to help design the next phase study, if applicable. All aspects of the Data Protection Act 1998 will be complied with. Any
information that would allow patients and clinicians (who are not part of the research team) to be identified will not be released into the public domain.

Archiving

Data relating to the trial will be securely archived for a minimum of 15 years.

Indemnity

As the study will be carried out by STH employees, NHS indemnity will apply as per the standard protocols. Indemnity for equipment will also be in place as the research will be conducted using equipment from a commercial company (Zilico).

11 Involvement of service users

We have involved members of the patient group Parathyroid UK in the development of the protocol and patient literature.

10 Methods for disseminating research results

After the completion of the study, a report will be written and will form part of the PhD thesis. The results of the study will be presented at relevant conferences and will be submitted for publication in a peer-reviewed journal.

11 Strategy for taking the work forward if the research project is productive

If the study demonstrates clear and consistent difference between the parathyroid and other soft tissue structures, a randomized controlled trial will be planned to demonstrate difference in clinical outcomes with the use of the device.

There will also be a need to recalibrate the device to enable interpretation of data in a user-friendly manner. The data generated from this project will be used for this purpose.

12 Intellectual Property arrangements

Pending meeting between Sheffield Teaching Hospitals and Zilico.
13 Costing the project

No extra patient contact or tests are required to conduct this study. The device will be on loan from Zilico for the purposes of this study. The project is being done as part of a student’s PhD. Stage 1 and 2 of the sterilisation processes have been completed and stage 3 will be done just prior to the start of the study. The costs for these stages and other consumables will be covered by a grant from the University of Sheffield Bioincubator (Research and Innovation Services).

14 Funding source

A grant (Collaborative R&D and Partnership Award) of £5000.00 has been awarded from The Sheffield Bioincubator (Research and Innovation Services) and the University of Sheffield.
A3.3 Ethics committee approval

Health Research Authority
NRES Committee Yorkshire & The Humber - Sheffield
Jarrow Business Centre
Viking Business Park
Rolling Mill Road
Jarrow
Tyne and Wear
NE32 3DT
Telephone: 0191 428 3561

18 August 2015

Mr Sabapathy Balasubramanian
Senior Clinical Lecturer and Honorary Consultant Surgeon
University of Sheffield
EU35, E Floor
Royal Hallamshire Hospital
Beech Hill Road
S10 2RX

Dear Mr Balasubramanian

Study title: Phase 1 study of Electrical Impedance Spectroscopy in Thyroid and Parathyroid Surgery

REC reference: 15/YH/0293
Protocol number: n/a
IRAS project ID: 168701

Thank you for your letter of 18 August 2015. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 16 July 2015.

Documents received

The documents received were as follows:

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant consent form</td>
<td>Version 1.1</td>
<td>22 July 2016</td>
</tr>
<tr>
<td>Participant information sheet (PIS)</td>
<td>Version 1.1</td>
<td>22 July 2016</td>
</tr>
</tbody>
</table>

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.
Yours sincerely

Kerry Dunbar
REC Assistant

E-mail: nrescommittee.yorkandhumber-sheffield@nhs.net

Copy to: Jemima Clarke, Sheffield Teaching Hospitals NHS Foundation Trust
A3.4 Participant information sheet

Participant Information Sheet
Development of a clinical protocol to use Electrical Impedance Spectroscopy during thyroid and parathyroid surgery

We would like to invite you to participate in a research study. Before you decide to take part, it is important that you understand why the research is being done and what is involved. This will help you make an informed decision. Please be assured that your clinic care will not be affected by your decision.

One of our team will go through this information sheet with you and answer any questions you may have. This should take around 15-30 minutes. Please take time to decide and discuss it with others if you wish. Please do not hesitate to ask us if there is anything that is unclear or if you would like further information.

Background to the study
The parathyroid glands are four tiny pieces of tissue, each about the size of a grain of rice. These glands are located on either side of the thyroid gland in the neck. They produce a hormone called PTH (Parathyroid Hormone). PTH helps maintain blood calcium levels. Without parathyroid glands, the blood calcium level would fall. This can produce a range of unpleasant symptoms including tingling, pins and needles, fatigue, painful muscle cramping and, if left untreated, seizures. There is also potential for long-term damage to the kidneys and bones.

During thyroid and parathyroid surgery, it is important to accurately identify parathyroid glands as they may sometimes be mistaken for other tissues in the neck. If the parathyroid glands are not identified during surgery, this may result in inadvertent damage to normal parathyroid glands or removal of tissue mistaken for a parathyroid gland.

This study aims to evaluate a new technique in the identification of parathyroid glands during surgery. If successful, the technique has potential to improve outcomes from these operations.

What do we do in the study?
A probe has been developed in Sheffield called ZedScan™. This probe may allow the surgeon to identify parathyroid gland tissue during surgery. It can signal the difference between tissue types during surgery due to the layout of the cells and the way electrical current travels through the tissues.

The probe is very safe and is currently used in clinical practice in some conditions in another department in Sheffield Teaching Hospitals.
Your surgery will be as planned except that we will use the probe during your operation to take readings from various tissues. This will take about 5-10 minutes. A photograph of the tissues may also be taken to aid analysis of the readings. Your surgery will then proceed as normal.

A specimen of tissue will be taken (a biopsy) and further readings will be taken from that before it is sent to the histology laboratory for tests.

**What are the possible benefits of taking part?**
You will not directly benefit from taking part in this study, but you will be helping others if the technology is found to help the surgeon identify parathyroid glands. Then, the risk to future patients of inadvertent injury during thyroid or parathyroid surgery will be reduced.

If the technology appears to work in this phase of the study, it will be further tested in another clinical trial to see if there is a reduction in the risk of complications from thyroid surgery and the failure rate following parathyroid surgery in the future.

**What are the risks of taking part?**
The probe used during the operation to make recordings is unlikely to influence the nature of the operation or the outcomes.

There may be a small increase in the duration of the operation time due to the need to take readings. This will not be for more than 10 minutes.

**Do I need to take part?**
No. Taking part is completely voluntary. It is up to you to decide whether or not to take part. If you decide to take part, you will be asked to sign a consent form. However, you are free to withdraw at any time. Whether you take part or not will not affect the standard of care you receive in any way at any time.

Obtaining informed consent for your planned operation is a separate process. This will occur in parallel to the consent for this research. A member of the surgical team responsible for your care will do this.

**What will happen to me if I decide to take part?**
You will have a discussion with a member of the research team. They will go through the details of the study and answer any questions you may have. You will then be asked to sign a consent form informing us that you agree to be involved. Three copies of this form will be made, of which one copy is for your records.

You will have your surgery as scheduled. During the surgery, the operation will pause for a few minutes while we take the readings with the probe. Then your operation will continue as normal. We will collect information from your biopsy results for the study and we may look at your blood results from your normal follow up appointment after surgery.

There are no extra hospital visits required for participation in this research project. There is no payment or vouchers for participation.
What if I do not wish to take part?
If you do not wish to take part, there will be no change in your management. Your doctor will still be responsible for making the best possible decision about your treatment.

What happens if I change my mind during the study?
You are free to withdraw at any time during the course of the study. This will not affect any treatment you might receive, now or in the future. If you decide to withdraw you just need to let us know. We would however like to keep any data that has already been collected.

Whom should I contact for further information?
Mr. Saba Balasubramanian, Senior Lecturer in Surgical Oncology, University of Sheffield (see below for details).

What if I wish to complain about the way in which this study has been conducted?
If you have any cause to complain about any aspect during the course of this study, the normal National Health Service complaints mechanisms are available to you. This process is not compromised in any way because you have taken part in a research study.

If you have any complaints or concerns, please contact Mr. Saba Balasubramanian (details provided below).

Alternatively, you can contact the Patient Services Team at Sheffield Teaching Hospitals on 0114 2712400 or via email at pst@sth.nhs.uk.

Or you can use the normal University of Sheffield complaints procedure and contact the Research & Consultancy Unit, University of Sheffield, 2/4 Palmerston Road, Sheffield, S10 2TE.

Am I entitled to compensation if something goes wrong?
In the event that something does go wrong, and you are harmed during the research there are no special compensation arrangements other than those normally open to you in your treatment in the National Health Service. If the harm is due to someone’s negligence, then you may have grounds for legal action for compensation against Sheffield Teaching Hospital NHS Foundation Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanism will still be available to you.

Will my taking part in this study be confidential?
Yes. All information gathered about you during the course of the research will be kept strictly confidential. Only the named researchers involved in the study handle data. Personal data (name, address etc.) will not be transferred or transmitted outside the hospital. Your GP however will be informed of your participation in this study.

What will happen to the results from this study?
The study results will be presented at learned conferences of medical societies and published in peer-reviewed scientific journals.

**Who is organizing and funding this study?**
The study is organized and carried out by the Department of Oncology at the University of Sheffield. The probe is being provided by a commercial company (Zilico). The study is being supported (funded) jointly by Zilico and the University of Sheffield.

**Contact name and numbers**
Mr. Saba Balasubramanian
General surgery directorate,
F Floor, Royal Hallamshire hospital,
Sheffield, S10 2JF
Telephone: 01142268392
Email: s.p.balasubramanian@sheffield.ac.uk
**A3.5 Participant consent**

Sheffield Teaching Hospitals NHS Foundation Trust

Mr S P Balasubramanian MS PhD FRCS, Senior Lecturer & Honorary Consultant Surgeon

Sarah Mason – Secretary to Mr Balasubramanian
Tel: 0114 271 1900
Fax: 0114 271 1901

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**CONSENT FORM**

<table>
<thead>
<tr>
<th>Patient Identification Number:</th>
<th>Date of Birth:</th>
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<tbody>
<tr>
<td>NHS/Hospital Number:</td>
<td>Initials:</td>
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</table>

**Title of Project:** Development of a clinical protocol to use Electrical Impedance Spectroscopy in thyroid and parathyroid surgery

**Chief Investigator:** Mr. Saba Balasubramanian

Please put your initials in each box if you agree with the corresponding statement.

1. I confirm that I have read and understood the participant information sheet for the above study [v.1.1 22 July 2015] and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. Data that has already been collected will be retained.

3. I understand that my clinical information and study data may be looked at by responsible individuals from the research staff, study sponsor (Sheffield Teaching Hospital NHS Foundation Trust) and from the regulatory authorities where it is relevant to my taking part in research; I give permission for these individuals to have access to my records.

4. I understand that medical photographs of the neck may be taken during the operation. These will be kept confidential but may be used anonymously in publication of the study findings. Patients will be unidentifiable from the photograph.

5. I understand that my medical information will be collected for use in this study and may be used to help develop new research. Data protection regulations will be observed and confidentiality will be maintained.

6. I agree to take part in the above study.

<table>
<thead>
<tr>
<th>Patient Signature</th>
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<tr>
<td>Patient name</td>
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<td>Investigator name</td>
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STH18736, Participant Consent Form, v1.1, 22Jul15
A3.6 Data collection form

Consent / Eligibility Visit

Date of Informed consent: 

Confirmation of Eligibility

Inclusion Criteria
- Undergoing thyroid or parathyroid surgery
- 18 years or over

Exclusion Criteria
- Undergoing re-do procedure to remove lymph nodes only
- Unable to understand spoken and written English
- Unable to give adequate informed consent

Gender

| Male | Female |

DOB

Group

| Total Thyroidectomy | Hemithyroidectomy | Isthmectomy | Bilateral neck exploration | Unilateral neck exploration | Targeted Parathyroidectomy |

Investigator confirming eligibility:

Signature

Name

Date
### Date of Surgery

Indication for surgery

Procedure

---

**Structures identified prior to use of probe:**

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<tr>
<th>Tissue</th>
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<th>Comments</th>
<th>Tissue</th>
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**BILATERAL NECK EXPLORATION**

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Histology

Thyroid:

Parathyroid:

Other:

Principal Investigator Signature Statement

I have reviewed this DCF and confirm that to the best of my knowledge, it accurately reflects the study information obtained for this participant. All entries were made by either myself or by a person under my supervision who has signed the Delegation Log.

Principal Investigator's Signature

Principal Investigator's Name

Date of Signature
A3.7 Raw data

Data sheets including raw and formatted data can be found in the online repository (DOI: 10.15131/shef.data.19161197).
A3.8 Graphical representation of the electrical impedance spectra over 14 frequencies produced from each tissue type in the central neck compartment

![Graphical representation of the electrical impedance spectra](image-url)
Normal Parathyroid

Abnormal Parathyroid
Muscle

Ex-Vivo Thyroid
Ex-Vivo Parathyroid

Frequency (Hz)

Impedance (Ohms)
A3.9 Normalising data – notes from recommendations from Prof. Brian Brown

Exclusions: Any spectra with negative values, any spectra where impedance rises with frequency

Duplicated spectra: If multiple spectra from the same tissue in the same participant was included, the mean of the spectra was used.

Spectra available to analyse:

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<tr>
<td>Ex-vivo Parathyroid</td>
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</table>

Normalised spectra: to remove the variability in amplitude and focus on the shape of the spectra

- Take the mean across a spectrum, divide each value by the mean to give 14 normalised values.
- Value/spectrum mean = normalised value

E.g. Patient 1 Frequency 1 (76 Hz)

\[
\frac{232.22}{199.42} = 1.164
\]
Create template: The median of the normalised spectra is the “template” of that tissue.

This provides the template to which other spectra can be compared.

Cervical templates were made from a cellular level. They were modelled in 3D based on histopathology/morphology of the tissues. Far more complex to do this in thyroid/parathyroid. Therefore the median of the spectra collected based on visual inspection is what we will use. Fairly reassured that the median and mean spectra are similar therefore there are few outliers, the spectra have a normal distribution around the mean and the shape of the curves produced are similar.

Template Matching: How closely does a spectrum compare to the template – deviation from the template expressed as a fraction (therefore between 0 and 1).

For each normalised value across a sectra

\[
\text{Normalised value} - \text{template value} \quad \text{Normalised value} + \text{template value}
\]

Take the absolute value (remove any negative signs)

Take the mean across the spectrum – this is the template match. The closer to 0 the value, the better the match.
Compare template matching:

How well the spectra for a tissue matches to a template can be compared by converting it to a relative probability. A threshold can be set and then it can be determined how many spectra would be matching at that threshold.

If the threshold was 0.01 for a template match, 10 parathyroid spectra and 2 thyroid spectra would match. This can be done over many thresholds to produce a graph of template matching.

If we look at the degree of template matching at each frequency – in this example thyroid compared to normal parathyroid template (NPT) and Normal Parathyroid compared to NPT – you can see that the thyroid template matches least well at the lower (76, 152, 305, 610, 1220 Hz)) and higher frequencies (19531, 39062, 78125 Hz). At these frequencies the normal parathyroid have a very close match to the template.
This is due to the shape of the curve being different. The thyroid spectra appear to cross the parathyroid spectra in the central portion of the curve, the parathyroid spectra stays flatter until the highest frequencies.
Therefore, when looking at the template match the match to the lowest and highest frequencies are the most important and these can be weighted. If we weight the matches from the frequencies 76, 152, 305, 610, 1220, 19531, 39062 and 78125 it changes the number of acceptable matches at each threshold. As there are different total numbers of spectra per tissue to compare the absolute value has been converted to a percentage. Here you can see that weighting the template match has made a bigger difference to the match of the thyroid compared to the parathyroid as those parts of the spectra have the most difference to the template. The amount of weighting could be changed, here I have used 4 times the value of the frequencies above and 1 times the value of all other frequencies.

\[ 4 \times (76 + 152 + 305 + 610 + 1220 + 19531 + 39062 + 78125) + 2441 + 4882 + 9765 + 156250 + 312500 + 625000 \]

Perhaps the change in template match dependent on weighting could be used in an algorithm to determine if a specimen is parathyroid.
Other things to consider: water would give very high values, if water had been used to clean the field before taking a reading this may change the amplitude of the spectra and explain why some are higher than others. Is this why fat has very high impedance because of high water content?

Saline would have a flat impedance.

Next steps:

Characteristic frequency

This is the frequency at which the steepest part of the curve is present. It may be a lower frequency in thyroids than parathyroids. The smaller the cells the higher the characteristic frequency – this would fit with parathyroid having smaller, more densely packed cells than thyroid tissue.

This together with the template matching may mean it is possible to separate parathyroid from other tissues.

ROC curves

The template match figures can be used to produce ROC by working out sensitivity and specificity at different thresholds.
A3.10 Normalised data

Normalised data used for analysis can be found in the online repository (DOI: 10.15131/shef.data.19161293)
A3.11 Normalised graphs

Normalised Thyroid Mean Impedance

![Graph for Normalised Thyroid Mean Impedance](image1)

Normalised Normal Parathyroid Mean Impedance

![Graph for Normalised Normal Parathyroid Mean Impedance](image2)
Normalised Visceral Fat Mean Impedance

Normalised Muscle Mean Impedance
Appendix 4: Appendices to Chapter 4

A4.1 Study protocol

TITLE: Assessment of quality of life in patients with long term Post-Surgical Hypoparathyroidism (PoSH)

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Sub-Investigators
Prof Nicola Brown, Department of Oncology and Metabolism, University of Sheffield

Study sponsor details
Sheffield Teaching Hospital NHS Foundation Trust

STH project reference number
STH20208

Protocol number, version and date
STH20208 Study Protocol_IRAS245001_07Jun18_v1.0
1 Research Hypothesis

Post-surgical hypoparathyroidism is associated with a significant detrimental impact on patients’ quality of life.

2 Abstract

Post-surgical hypoparathyroidism is an important side effect of thyroid and parathyroid surgery. Given the increasing incidence of thyroid cancer and the rising rates of thyroid surgery, this condition is expected to occur more frequently. Long term Post-Neurosurgical Hypoparathyroidism (PoSH) is defined as the need for calcium and/or vitamin D supplements to prevent or treat hypoparathyroidism and/or hypocalcaemia lasting for beyond 6 months after surgery. Although detrimental effects on renal function and other organs have been demonstrated, the impact on patients’ quality of life is poorly documented. It has been suggested that despite medical treatment, all domains of quality of life are affected in patients with PoSH. This study aims to do a detailed assessment of symptoms and quality of life in different cohorts of patients with post-surgical hypoparathyroidism and compare these patients with similar cohorts of patients without hypoparathyroidism.

3 Background: clinical and scientific justification

Intraoperative bruising, devascularisation or inadvertent excision of the parathyroid glands can lead to low levels of parathyroid hormone (PTH) post-operatively causing hypocalcaemia. This can have significant side effects on the patient if left untreated including death. The condition is managed with calcium and/or activated vitamin D supplements. In most patients hypocalcaemia is transient and medication is stopped within six months but for some it can be permanent.

The incidence of post thyroidectomy hypocalcaemia and/or hypoparathyroidism varies greatly in published literature. Studies report that anywhere between 19-38% of patients develop hypocalcaemia (Edafe, Antakia et al. 2014). Discrepancies between the reported figures are attributed to the use of different definitions of hypocalcaemia and hypoparathyroidism and the inclusion of all thyroid surgery, some of which do not predispose to hypocalcaemia. In the UK, the fourth national audit by
the British Association of Endocrine and Thyroid Surgeons (BAETS) reported a 25% incidence of transient hypocalcaemia and a 12% incidence of long term or permanent hypocalcaemia after bilateral thyroid surgery. The prevalence of hypoparathyroidism is an estimated 37 per 100 000 person-years in the United States and 22 per 100 000 person-years in Denmark (Clarke, Brown et al. 2016). A recent study in the UK has shown prevalence of postsurgical hypoparathyroidism to be 23 per 100,000 (Vadiveloo, Donnan et al. 2017).

A number of predictive factors for post-surgical hypoparathyroidism exist; perioperative PTH, preoperative vitamin D and postoperative changes in calcium are biochemical predictors of post-thyroidectomy hypocalcaemia. Clinical predictors include female gender, Graves’ disease, need for parathyroid auto-transplantation and inadvertent excision of PTGs (Edafe, Antakia et al. 2014).

Immediate postoperative hypocalcaemia causes a wide variety of symptoms including peri-oral and digital paraesthesiae, tetany, laryngospasm, ECG changes and arrhythmia and even seizures. Although this can be treated medically, hypocalcaemia also results in a longer post-operative hospital stay. The median hospital stay after total thyroidectomy was two days (Chadwick 2012). Patients staying longer than two days had a significantly higher rate of hypocalcaemia compared to those discharged within two days (Chadwick 2012). Permanent hypoparathyroidism requires long-term treatment but is also associated with other significant morbidity including renal stones, nephrocalcinosis and soft tissue calcification (Khan, Waguespack et al. 2011). In addition, there appears to be a significant impact on quality of life (Cho, Moalem et al. 2014), but this has not been studied in detail.

4 Aim of study

A recent systematic review concluded that further studies are required that quantify the effect of hypoparathyroidism on patients’ QoL including aetiology and other related factors (Buttner, Musholt et al. 2017). The aim of the study is to perform a detailed assessment of symptoms and quality of life in different cohorts of patients with post-surgical hypoparathyroidism and compare these patients with similar cohorts of patients without hypoparathyroidism. This study will take into account the
aetiology of hypoparathyroidism, treatment and co-morbidities to analyse for possible predictive factors.

5 Plan of investigation

Summary of study design

This is a cross-sectional observational study.

Setting for the project

Online questionnaire

Participants

Participants with post-surgical hypoparathyroidism and appropriate controls (those without PoSH) will be identified from four different sources:

a. Butterfly Thyroid Cancer Trust (BTCT) – This is a UK national patient charity whose members have been diagnosed with thyroid cancer. The vast majority of patients will have had thyroid surgery, with a small proportion having PoSH as a result of surgery and the remainder will serve as a pool from which to recruit control patients

b. Association of Multiple Endocrine Neoplasia Disorders (AMEND) – This is a UK national patient charity for patients with MEN disorders. A significant proportion of patients/members of this charity will have had thyroid and/or parathyroid surgery, with some having PoSH. Participants for both the disease and control groups will therefore be recruited from this membership.

c. Patients who have undergone surgery for benign or malignant thyroid disease or for primary hyperparathyroidism in the Sheffield endocrine surgery units from 2010 to 2016 will be screened. Patients who have ongoing PoSH will be recruited to take part in the study. A random selection of age, sex and ‘year of surgery’ matched
patients without PoSH will be identified from the operating theatre database to serve as controls.

d. Patients with thyroid cancer and MEN1 or MEN2 syndrome attending the follow up thyroid cancer clinics at Weston Park hospital and the endocrine clinics at the Royal Hallamshire Hospital in Sheffield will be invited to take part as participants in the ‘disease’ and ‘control’ groups respectively.

Sample size

Inclusion criteria

Patients who have had thyroid and/or parathyroid surgery

Exclusion criteria –

Patients below the age of 18

Patients who do not speak or understand English

Patients with learning difficulties

Patients unwilling to take part

The study aims to receive at least 100 completed surveys.

Recruitment

Members of the AMEND and BTCT charities will be invited to participate in the study via their websites, newsletters and social media. A link to the online survey will be available on the invitation. Potential participants identified from the MEN and Thyroid cancer clinic lists will be invited to take part in the survey. This will be by a number of different methods deemed appropriate by the researcher – email, post, telephone and face to face in the clinic. The invitation will include brief details of the study, how to access the survey, approximate time taken to complete the survey and other relevant information. Once the questionnaire has been accessed a more detailed participant information sheet will be presented followed by a consent page which
must be completed to access the questionnaire. The questionnaire will include
demographic details, details on surgery and treatment for hypoparathyroidism, a
QoL survey (modified SF-36 quality-of-life measure), questions on symptoms of
hypoparathyroidism (including the hypocalcaemia symptom score - HSS) and other
questions relating to patients’ perceptions of the impact of the diagnosis. An email
address will be provided for patients to request a paper copy of the questionnaire.
Patients will have 8 weeks to complete the questionnaire and two reminders will be
sent out before the end of the 8-week period. The questionnaire will be available via
the REDCap website and can be accessed via computer, mobile or tablet devices that
are linked to the Internet. REDCap is a secure web application for conducting online
questionnaires.

Outcome measures

Primary outcome: Score SF-36 in the two groups

Secondary outcomes will include the HSS. The outcomes will be stratified by
pathology (benign thyroid disease, non-syndromic thyroid cancer, sporadic primary
hyperparathyroidism, syndromic primary hyperparathyroidism) and time since onset
of PoSH/surgery (<5 years, 5-15 years and >15 years): depending on the adequacy and
quality of available data.

Statistical analysis

Given the exploratory nature of the study, the analyses will primarily be descriptive.
Categorical, binary and ordinal data will be reported as frequencies or percentages.
Normally distributed continuous data will be described using mean and standard
deviation (SD). Continuous data that is not normally distributed will be described
using median and inter-quartile range (IQR). Patients comprise of participants with
long term hypoparathyroidism and control cohorts will be those without
hypoparathyroidism and those with transient hypoparathyroidism. Depending on the
numbers available, these groups may be further subdivided as per the underlying
condition – non-syndromic thyroid cancer, benign thyroid disease, non-syndromic
parathyroid disease and patients with MEN syndromes.
The demographic features, symptom and QoL scores will be compared in patients and controls (and within specified subgroups if possible) and any observed differences will be tested for statistical significance. The statistical methods used will depend on the type of data (categorical, ordinal, normal continuous, non-normal continuous) and the number of groups (which depend on the numbers available). Given the exploratory nature of the dataset, it is likely that only univariate methods will be employed.

6 Justification of use of screening tools

The sf-36 and HSS are validated quality of life tools.

7 Project management

The chief investigator will be responsible for managing online replies and any paper copy requests. They will liaise with the charities distributing the survey.

Expertise of the researcher and associated team

The PI has a good relationship with the two charities who are on board with this study and are happy to approach their members. The PI also has a database of patients within Sheffield who are able to be approached to participate.

8 Funding

This project is unfunded.

9 Ethical issues

Participant information will be kept confidentially at all times and no patient identifiable data will be presented. Participants will consent to participating in the study at the start of the survey. They will not have access to the survey unless they have consented. Patients will have the option to participate or not and will be able to leave the questionnaire at any point should they wish to. They do not need to provide identifiable information to participate but can choose to do.

Involvement of service users
SF-36 and HSS are widely accepted tools by patient groups for measuring quality of life.

10 Dissemination

Results from this study will be written up as a report and submitted to a peer reviewed journal for publication. The report and a lay summary of the results will be distributed to the charities involved in the study for dissemination to their members. The participants from STH will be informed that they can go to the charity websites to see the results of the study.

11 Taking the work forward

This will provide a basis for understanding the scope of PoSH and provide evidence to validate novel methods to reduce the rate.
A4.2 Ethical approval

East of Scotland Research Ethics Service (EoSRES)

Mrs Sarah Hillary
Clinical Research Associate
Sheffield Teaching Hospitals NHS Foundation Trust
FU03
The Medical School, University of Sheffield
Beech Hill Road
S10 2RX

Date: 16 July 2018
Your Ref: LR/18/ES/0091
Out Ref: 0
Enquiries to: Mrs Lorraine Reilly
Direct Line: 01382 383878
Email: esres.tayside@nhs.net

Dear Mrs Hillary

Study title: Assessment of quality of life in patients with long term Post-Surgical Hypoparathyroidism (PoSH)
REC reference: 18/ES/0091
Protocol number: 0
IRAS project ID: 245001

The Proportionate Review Sub-Committee of the East of Scotland Research Ethics Service REC 2 reviewed the above application on 16 July 2018.

Provisional opinion

The Sub-Committee would be content to give a favourable ethical opinion of the research, subject to clarification of the following issues and/or the following changes being made to the documentation for study participants:

1. The Committee requested clarification regarding the statistical review, as A56 on the IRAS form states that no statistical review has been performed; however, the protocol states that tests for statistical significance between various outcomes and subgroups would be performed. The Committee also requested clarification as to whether a statistician has been involved as per the Scientific Review forms and if not they suggested that it would be helpful to discuss with a statistician or have a statistician involved.

2. The Committee noted that there would be no sample size calculation performed; however, the IRAS form states that 100 patients have been deemed to be sufficient and requested clarification as to how the sample size was decided.

3. The Committee was also unclear as to how the study team could control that only 100 participants would be included in the study and whether the unstructured invitation approach could ensure that there was balance between control and PoSH groups.

4. The Committee noted that the charity mail invitation and reminder stated ‘We would be very grateful if any patient who has had thyroid or parathyroid surgery could
complete the survey, regardless of whether they themselves have ever had low blood calcium levels’. The Committee requested clarification as to whether the charity would provide patient contacts to the research team or whether the invitation was aimed at the potential participants themselves.

5. Further information was requested as to how non-cancer patients would be identified and recruited.

6. The Committee noted that page 8 in the protocol states that ‘They do not need to provide identifiable information to participate; but, are asked to provide their name, gender, data of birth, telephone number, email address and GP practice name. There was no advice provided in the information sheet and consent form informing potential participants that providing demographic data was optional. The Committee requested further clarification as to why demographic information was required, and whether it would be linked to the study data.

7. The Committee also requested clarification as to what medical information would be requested from the GP; whether the data would be anonymised; who from the study team would have access to the data and where it would be stored.

8. The Committee requested clarification as what was meant by a ‘random selection’ of ‘matched patients’.

9. The Committee requested clarification as to how the study team could ensure that the participant had adequate English language/reading understanding.

10. The Committee requested further information regarding the security measures in place for participants accessing the online survey.

11. The Committee requested clarification as to whether or not consent had been obtained to use the operating theatre database to contact patients for the control group.

12. The Committee requested that the sentence in the STH Invitation letter was reviewed as it was confusing ‘We would be very grateful if you have had thyroid or parathyroid surgery could complete the survey, regardless of whether you have ever had low blood calcium levels’.

13. The Committee requested that information was included in the covering letter informing potential participants how the research team obtained their details to contact them.

14. The Committee requested that more information was included in the information sheet regarding the study, as it was quite vague.

15. If applicable, insert information in the information sheet as to who outside the research team may have access to participant’s identifiable data.

16. The Committee noted that the information sheet stated that should participants encounter any problems to contact Sarah Hillary; however, there was no information as to who the person was and her role within the study.

17. The Committee requested that the information sheet included information as to who to contact should participants require further information about the study and how participants could obtain a copy of the study results.
18. Adapt the following paragraph in the information sheet and insert under the heading ‘Who has reviewed this study?’

‘The East of Scotland Research Ethics Service REC 2, which has responsibility for scrutinising all proposals for medical research on humans, has examined the proposal and has raised no objections from the point of view of research ethics. It is a requirement that your records in this research, together with any relevant medical records, be made available for scrutiny by monitors from << name of sponsor company (if appropriate)>> and NHS <<insert name of Health Board/Trust>>, whose role is to check that research is properly conducted and the interests of those taking part are adequately protected.’

Suggestions/recommendations

- The Committee noted that the IRAS form stated that survey questions could be skipped and suggested that a box was added ‘Do not want to answer’ for participants to click.

- The Committee noted that some of the survey questions could be potentially disturbing for participants to answer; for example, the impact of their emotional and physical activates and suggested that information could be added to the end of the survey informing participants about support if required.

When submitting a response to the Sub-Committee, the requested information should be electronically submitted from IRAS. Please refer to the following guidance for instructions on how to submit a response to provisional opinion electronically from IRAS: https://www.myresearchproject.org.uk/help/hlptheticalreview.aspx#After-submit-to-REC

Please submit revised documentation where appropriate underlining or otherwise highlighting the changes which have been made and giving revised version numbers and dates. You do not have to make any changes to the REC application form unless you have been specifically requested to do so by the REC.

Authority to consider your response and to confirm the final opinion on behalf of the Committee has been delegated to the Alternate Vice-chair.

Please contact Mrs Lorraine Reilly, REC Manager (details at top of letter) if you need any further clarification or would find it helpful to discuss the changes required with the lead reviewer.

The Committee will confirm the final ethical opinion within 7 days of receiving a full response. A response should be submitted by no later than 15 August 2018.

Social or scientific value; scientific design and conduct of the study

The Committee requested clarification regarding the statistical review, as A56 on the IRAS form stated that no statistical review had been performed; however, the protocol stated that tests for statistical significance between various outcomes and subgroups would be performed. The Committee requested clarification as to whether a statistician had been involved as per the Scientific Review forms and if not they suggested that it would be helpful to discuss with a statistician or have a statistician involved.

The Committee noted that there would be no sample size calculation performed; however, the IRAS form stated that 100 patients had been deemed to be sufficient and requested clarification as to how the sample size was decided.
The Committee was also unclear as to how the study team could control that only 100 participants would be included in the study and whether the unstructured invitation approach could ensure that there was balance between control and POSH groups.

**Recruitment arrangements and access to health information, and fair participant selection**

The Committee noted that the charity mail invitation and reminder stated ‘We would be very grateful if any patient who has had thyroid or parathyroid surgery could complete the survey, regardless of whether they themselves have ever had low blood calcium levels’. The Committee requested clarification as to whether the charity would provide patient contacts to the research team or whether the invitation was aimed at the potential participants themselves.

The Committee requested further information as to how non-cancer patients would be identified and recruited.

**Favourable risk benefit ratio; anticipated benefit/risks for research participants (present and future)**

The Committee noted that page 8 in the protocol stated that ‘They do not need to provide identifiable information to participate; but, are asked to provide their name, gender, data of birth, telephone number, email address and GP practice name. There was no advice provided in the information sheet and consent form informing potential participants that providing demographic data was optional. The Committee requested further clarification as to why demographic information was required, and whether it would be linked to the study data.

The Committee also requested clarification as to what medical information would be requested from the GP; whether the data would be anonymised; who from the study team would have access to the data and where it would be stored.

**Care and protection of research participants; respect for potential and enrolled participants' welfare and dignity**

The Committee requested clarification as what was meant by a ‘random selection’ of ‘matched patients’.

The Committee requested clarification as to how the study team could ensure that the participant had adequate English language/reading understanding.

The Committee requested further information regarding the security measures in place for participants accessing the online survey.

**Informed consent process and the adequacy and completeness of participant information**

The Committee requested clarification as to whether or not consent had been obtained to use the operating theatre database to contact patients for the control group.

**Suitability of supporting information**

The Committee requested that the sentence in the STH Invitation letter was reviewed as it was confusing ‘We would be very grateful if you have had thyroid or parathyroid surgery could complete the survey, regardless of whether you have ever had low blood calcium levels’.
The Committee requested that information was included in the covering letter informing potential participants how the research team obtained their details to contact them.

The Committee noted that the IRAS form stated that survey questions could be skipped if required and suggested that a box was added ‘Do not want to answer’ for participants to click.

The Committee noted that some of the survey questions could be potentially disturbing for participants to answer; for example, the impact of their emotional and physical activates and suggested that information could be added to the end of the survey informing participants about suitable support.

The Committee requested that more information was included in the information sheet regarding the study, as it was quite vague.

If applicable, insert information in the information sheet as to who outside the research team may have access to participant’s identifiable data.

The Committee noted that the information sheet stated that should participants encounter any problems to contact Sarah Hillary; however, there was no information as to who the person was and her role within the study.

The Committee requested that the information sheet included details regarding who to contact should participants require further information about the study and how participants could obtain a copy of the study results.

**Documents reviewed**

The documents reviewed were:

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copies of advertisement materials for research participants [Advertisement]</td>
<td>1.0</td>
<td>21 June 2018</td>
</tr>
<tr>
<td>Copies of advertisement materials for research participants [Reminder email]</td>
<td>1.0</td>
<td>05 July 2018</td>
</tr>
<tr>
<td>Covering letter on headed paper [Cover Letter (Version date 21/6/2018)]</td>
<td>1.0</td>
<td>28 June 2018</td>
</tr>
<tr>
<td>IRAS Application Form [IRAS_Form_05072018]</td>
<td></td>
<td>29 June 2018</td>
</tr>
<tr>
<td>IRAS Checklist XML [Checklist_05072018]</td>
<td></td>
<td>05 July 2018</td>
</tr>
<tr>
<td>Letters of invitation to participant [Invitation]</td>
<td>1.0</td>
<td>21 June 2018</td>
</tr>
<tr>
<td>Non-validated questionnaire [Survey]</td>
<td></td>
<td>21 June 2018</td>
</tr>
<tr>
<td>Participant consent form [Consent]</td>
<td></td>
<td>21 June 2018</td>
</tr>
<tr>
<td>Participant information sheet (PIS) [PIS]</td>
<td></td>
<td>21 June 2018</td>
</tr>
<tr>
<td>Referee’s report or other scientific critique report [Scientific Review]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Referee’s report or other scientific critique report [Scientific Review]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Research protocol or project proposal [Protocol]</td>
<td>1.0</td>
<td>21 June 2018</td>
</tr>
<tr>
<td>Summary CV for Chief Investigator (CI) [CV Mrs Hillary]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summary CV for supervisor (student research) [CV Dr Balasubramanian]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summary CV for supervisor (student research) [CV Dr Brown]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Membership of the Committee

The members of the Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

18/ES/0091 Please quote this number on all correspondence

Yours sincerely

pp
Ms Petra Rauchhaus
Alternate Vice-chair

Email: eosres.tayside@nhs.net

Enclosures: List of names and professions of members who took part in the review

Copy to: Dr Modhumita Harris, Sheffield Teaching Hospitals NHS FT
A4.3 Participant Invitation

Dear Sir/Madam,

We are constantly trying to find ways to improve and make surgery safer. As you have previously had surgery at the Endocrine Surgery Unit we would like to invite you to participate in a study by the University of Sheffield and Sheffield Teaching Hospitals that aims to determine patients’ quality of life following surgery for either thyroid or parathyroid disease. The survey will take 10 minutes to complete.

The focus of this study is to look in depth at the effect low blood calcium levels, one of the potential side effects of this type of surgery, has on quality of life. Our research group is working on new ways of reducing this side effect of surgery so we want to show what effect this side effect has on patients.

We would be grateful if you could complete the survey if you have had thyroid or parathyroid surgery. Although we are looking at low blood calcium, you do not need to have had this to complete the survey.

To participate in the survey please use the link below:
https://redcap.shef.ac.uk/surveys/?s=PYKF74KHFF

For further information or to be sent a paper copy of the questionnaire please contact Mrs Sarah Hillary, Clinical Research Associate and Chief Investigator of the project (s.l.hillary@sheffield.ac.uk).

The results of the study will be available via www.butterfly.org.uk or www.amend.org.uk.

Thank you

Mr Saba Balasubramanian
Consultant Surgeon and Honorary Senior Lecturer
Endocrine Surgery
Sheffield Teaching Hospitals NHS Foundation Trust
Quality of Life following Thyroid and Parathyroid Surgery

Study Title: Assessment of Quality of Life in patients with long term Post-Surgical Hypoparathyroidism (PoSH)

We are constantly trying to find ways to improve and make surgery safer. Our research group is working on new ways of reducing hypocalcaemia after surgery so we want to show what effect this complication has on patients.

Study Information

Low blood calcium or hypocalcaemia can be a problem for patients. One reason for the calcium being low is a condition called hypoparathyroidism. This condition is due to reduced activity or function of parathyroid glands - these are tiny glands in the neck (two on either side), each about the size of a grain of rice - which control the levels of calcium in the blood. In most patients, the reduced activity or loss of function is a result of having neck surgery. During neck surgery one or more of these glands can become bruised, damaged or even accidentally removed. This increases the risk of getting lower levels of parathyroid hormone, which results in low blood calcium levels.

Hypoparathyroidism after surgery may lead to long-term negative effects on health. Although this has been recognised, the extent of these effects and their impact on patients has not been studied in great detail. This study aims to understand the quality of life of patients who have had thyroid and/or parathyroid surgery and who may or may not have this condition as a result of the operation. This study will support ongoing research into ways to reduce this side effect and make surgery safer.

We are therefore looking for volunteers who have had a neck operation for thyroid and/or parathyroid disease to complete a survey, whether or not you have ever had low blood calcium. The survey will ask you about your operation and your general health and then ask a series of questions to see how the surgery has affected you.

How long will it take to complete?

The questionnaire will take less than 10 minutes to complete.

How will my responses be kept?

All answers given will be kept confidential and any data provided will be anonymised. The data sent online is encrypted and kept secure at all times.

Why have we asked for personal information?

We have asked for your personal information as part of the survey. This allows us to group your responses, for example, according to age. If you provide your contact information and you were uncertain of how to answer some of the clinical information in the study the researchers will contact you or your GP to clarify the information you were unable to provide yourself. Any personal information provided will not be passed on to any third parties and will be kept securely. Your responses will be anonymised once the data is collected.

Sheffield Teaching Hospitals NHS Foundation Trust (STH) may use your name, and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from STH and regulatory organisations may need to access your medical records for the purposes of research and patient care.
research records to check the accuracy of the research study. The only people in STH who will have access to information that identifies you will be people who need to contact you to audit the data collection process. The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number or contact details. STH will keep identifiable information about you from this study for 10 years after the study has finished.

What if I do not wish to provide this?

If you do not wish to provide this information then you may still complete the rest of the questionnaire as an anonymous response and it will not affect your inclusion in the study.

Do I have to answer all the questions?

If there is a question you would prefer not to answer then you are able to skip the question and continue on to the rest of the survey. Participation is voluntary and you can withdraw from the study at any point. You may choose not to answer all of the questions in the survey.

Who has reviewed this study?

The East of Scotland Research Ethics Service REC 2, which has responsibility for scrutinising all proposals for medical research on humans, has examined the proposal and has raised no objections from the point of view of research ethics. It is a requirement that your records in this research, together with any relevant medical records, be made available for scrutiny by monitors from Sheffield Teaching Hospitals NHS Foundation Trust, whose role is to check that research is properly conducted and the interests of those taking part are adequately protected.

Integrated Research Application System (IRAS) Number: 245001

Who can I contact from the study?

If you have any problems completing the survey, would like a paper copy to complete or just need to get in touch then please contact Mrs Sarah Hillary, Clinical Research Associate and Chief Investigator for the study. s.i.hillary@sheffield.ac.uk

Where will the results be available?

The results of the study will be made available at www.butterfly.org.uk or www.amend.org.uk

GDPR - Transparency Statement

Sheffield Teaching Hospitals NHS Foundation Trust (STH) is the sponsor for this study based in the United Kingdom. We will be using information from you in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. STH will keep identifiable information provided by you for 10 years after the study has finished.
Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

You can find out more about how we use your information www.sheffieldclinicalresearch.org

<table>
<thead>
<tr>
<th>I confirm I have read the information provided above about the study and understand that participation in this survey is voluntary, data protection regulations will be observed and confidentiality will be maintained.</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ ] Yes</td>
</tr>
<tr>
<td>[ ] No</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>I wish to participate in the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ ] Yes</td>
</tr>
<tr>
<td>[ ] No</td>
</tr>
</tbody>
</table>
### Demographics

<table>
<thead>
<tr>
<th>Name</th>
<th>(You may leave this blank if you do not wish to provide this information)</th>
</tr>
</thead>
</table>
| Gender | ○ Female  
        ○ Male  
        ○ Do not wish to disclose |
| Date of birth | (You may leave this blank if you do not wish to provide this information) |
| If we require further information or clarification may we contact you or your GP? | ○ Yes  
        ○ No |
| Your telephone number (include area code) | (You may leave this blank if you do not wish to provide this information, it will only be used to clarify your responses) |
| Your email | (You may leave this blank if you do not wish to provide this information, it will only be used to clarify your responses) |
| Practice Name | (You may leave this blank if you do not wish to provide this information, it will only be used to clarify your responses) |
Your Surgery

The thyroid is the butterfly shaped gland that is located in the neck. It is surrounded by four small parathyroid glands. You can have surgery to remove the thyroid gland, one or more parathyroid glands or a combination of the two. Sometimes there is inadvertent (unplanned) removal of parathyroid glands with the thyroid. The parathyroid glands can also be bruised or have their blood supply damaged as a result of surgery. The parathyroid glands regulate the amount of calcium in your blood.

Have you had thyroid or parathyroid surgery?  
☐ Thyroid  
☐ Parathyroid  
☐ Both  
☐ Unsure

Do you have MEN (Multiple Endocrine Neoplasia) syndrome?  
☐ Yes  
☐ No  
☐ Unsure

Whereby it has been demonstrated that your thyroid and/or parathyroid condition was a result of a genetic change.

What year did you have surgery on your thyroid?  
(If you had more than one operation, write down the year of the last operation)

Why did you have surgery on your thyroid?  
☐ Thyroid nodule or goitre or lump proven to be benign  
☐ Thyroid cancer  
☐ Graves’ disease / hyperthyroidism  
☐ Unsure

How many thyroid operations have you had in total?

As far as you have been made aware, how much of the thyroid gland was removed in all the operations you have had so far?  
☐ Most or all of the thyroid (subtotal or near-total or total thyroidectomy)  
☐ Part of the thyroid (isthmectomy or hemi-thyroidectomy)  
☐ Unsure
<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did you have any other treatment/therapies after thyroid surgery?</td>
<td>☐ Radiiodine&lt;br&gt;☐ Radiotherapy&lt;br&gt;☐ Ongoing thyroxine treatment&lt;br&gt;☐ None&lt;br&gt;☐ Unsure</td>
</tr>
<tr>
<td>If you had surgery for thyroid cancer, do you know if your current thyroxine treatment is high or normal dose?</td>
<td>☐ High dose&lt;br&gt;☐ Normal dose&lt;br&gt;☐ Not sure</td>
</tr>
<tr>
<td>Some patients with thyroid cancer are advised to have 'high dose' thyroxine treatment</td>
<td></td>
</tr>
<tr>
<td>What year did you have parathyroid surgery?</td>
<td>(If you had more than one operation, write down the year of the last operation)</td>
</tr>
<tr>
<td>How many parathyroid operations have you had so far?</td>
<td></td>
</tr>
<tr>
<td>How many parathyroid glands were removed in all of the parathyroid operations you have had so far?</td>
<td></td>
</tr>
<tr>
<td>Did you have surgery on both or just one side of the neck?</td>
<td>☐ Both sides&lt;br&gt;☐ One side&lt;br&gt;☐ Unsure&lt;br&gt;☐ (If you have had more than one operation, please take into account all parathyroid operations)</td>
</tr>
<tr>
<td>Do you currently have persistence of the disease/condition for which you have had thyroid and/or parathyroid surgery?</td>
<td>☐ Yes&lt;br&gt;☐ No&lt;br&gt;☐ Unsure</td>
</tr>
<tr>
<td>Have you had treatment for hypocalcaemia (low blood calcium) after your thyroid and/or parathyroid operation?</td>
<td>☐ Never&lt;br&gt;☐ Temporarily&lt;br&gt;☐ Currently taking&lt;br&gt;☐ Don't know</td>
</tr>
<tr>
<td>You need not take into account any calcium and/or vitamin D tablets you may be taking for other conditions e.g. osteoporosis</td>
<td></td>
</tr>
<tr>
<td>How long did you take/have you been taking the calcium and/or vitamin D medications for?</td>
<td>☐ Less than 6 weeks&lt;br&gt;☐ Less than 6 months&lt;br&gt;☐ More than 6 months&lt;br&gt;☐ Don't know</td>
</tr>
<tr>
<td>If you had blood tests in the last six months, was your most recent blood calcium level</td>
<td>☐ High&lt;br&gt;☐ Normal&lt;br&gt;☐ Low&lt;br&gt;☐ Very low&lt;br&gt;☐ Not Sure&lt;br&gt;☐ Did not have a test in the last 6 months</td>
</tr>
</tbody>
</table>
If you had blood tests in the last six months was your most recent blood PTH (parathyroid hormone) level:
- High
- Normal
- Low
- Very low
- Not sure
- Did not have a test in the last 6 months

Please list all of your current medication and doses:

_________________________________________________________
Please answer the following questions in response to how you are feeling today unless otherwise specified.

<table>
<thead>
<tr>
<th>In general would you say your health is</th>
<th>Excellent</th>
<th>Very good</th>
<th>Good</th>
<th>Fair</th>
<th>Poor</th>
</tr>
</thead>
</table>

| Compared to one year ago, how would you rate your health in general now? | Much better than one year ago | Somewhat better than one year ago | About the same | Somewhat worse than one year ago | Much worse than one year ago |
The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

<table>
<thead>
<tr>
<th>Activity</th>
<th>Limited a lot</th>
<th>Limited a little</th>
<th>Not limited at all</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vigorous activities e.g. running, lifting heavy objects, participating in strenuous sports</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Moderate activities e.g. moving a table, pushing a vacuum cleaner, bowling or playing golf</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Lifting or carrying groceries</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Climbing several flights of stairs</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Climbing one flight of stairs</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Bending, kneeling or stooping</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Walking more than one mile</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Walking several blocks</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Walking one block</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Bathing or dressing yourself</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Problem</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>--------------------------------------------------------------------------------------------</td>
<td>-----</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>Cut down the amount of time you spent on work or other activities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accomplished less than you would like</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Were limited in the kind of work or other activities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Had difficulty performing the work or other activities (e.g., it took extra effort)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Problem</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>-----------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>Cut down the amount of time you spent on work or other activities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accomplished less than you would have liked</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Didn't do work or other activities as carefully as usual</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours or groups?</strong></td>
<td>Not at all</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slightly</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quite a bit</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extremely</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Very severely</td>
<td></td>
<td></td>
</tr>
<tr>
<td>How much bodily pain have you had during the past 4 weeks?</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Very mild</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Very severe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>During the past 4 weeks, how much did pain interfere with your normal work?</td>
<td>Not at all</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Including both work outside the home and housework</td>
<td>A little bit</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderately</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quite a bit</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extremely</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
These questions are about how you feel and how things have been with you during the last 4 weeks. For each question, please give the answer that comes closest to the way you have been feeling.

<table>
<thead>
<tr>
<th>How much of the time during the past 4 weeks....</th>
<th>All of the time</th>
<th>Most of the time</th>
<th>A good bit of the time</th>
<th>Some of the time</th>
<th>A little bit of the time</th>
<th>None of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did you feel full of vigor or enthusiasm?</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Have you been a very nervous person?</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Have you felt so down in the dumps that nothing could cheer you up?</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Have you felt calm and peaceful?</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Did you have lots of energy?</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Have you felt downhearted and blue?</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Did you feel worn out?</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Have you been a happy person?</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Did you feel tired?</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

During the past 4 weeks, how much time has your physical health or emotional problems interfered with your social activities? (e.g. visiting friends, relatives etc.)

○ All of the time
○ Most of the time
○ Some of the time
○ A little bit of the time
○ None of the time
<table>
<thead>
<tr>
<th>Statement</th>
<th>Definitely true</th>
<th>Mostly true</th>
<th>Don’t know</th>
<th>Mostly false</th>
<th>Definitely false</th>
</tr>
</thead>
<tbody>
<tr>
<td>I seem to get sick a little easier than other people</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>I am as healthy as anybody I know</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>I expect my health to get worse</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>My health is excellent</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>
## During the past 4 weeks, have you had any of the following problems?

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Almost never</th>
<th>Sometimes</th>
<th>Often</th>
<th>Always</th>
<th>Don't know</th>
</tr>
</thead>
<tbody>
<tr>
<td>I get tingling ('pins and needles') in my fingers, toes or around my mouth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I get numbness in my fingers, toes or around my mouth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I get muscle cramps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I have diarrhoea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>My brain feels &quot;foggy&quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I have dry skin or hair</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Don't know</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-----</td>
<td>-----</td>
<td>------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart problems</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cataracts or other eye problems</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dental problems</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney stones</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney failure</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epilepsy/fits</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Thank you for completing the survey.

The results will be available through www.butterfly.org.uk and www.amend.org.uk.

If this survey has raised concerns regarding your health, please discuss this with your GP or hospital Consultant.
A4.5 Participant responses

A spreadsheet of participant responses and formatted scores can be found on the online repository (DOI: 10.15131/shef.data.19161470).
Appendix 5: Appendices to Chapter 5

A5.1 Management protocol for asymptomatic post-operative thyroid and parathyroid patients in Sheffield Teaching Hospitals NHS Foundation Trust

![Management protocol diagram]

- **Thyroidectomy**
  - **PTH < 2.0**
    - **Ca\(^{2+} < 2.1****: 1-α-calcitriol 1 μg OD, Oral calcium 1g tds, Review ~ day 5
    - **Ca\(^{2+} ≥ 2.1****: 1-α-calcitriol 0.5 μg OD, Oral calcium 1g tds, Review ~ day 5
  - **PTH ≥ 2.0**
    - **Ca\(^{2+} < 2.1****: Oral calcium 1g tds, Review ~ day 5
    - **Ca\(^{2+} ≥ 2.1****: No treatment required

- **Parathyroidectomy**
  - **PTH < 1.6**
    - Oral calcium 1g tds, Discuss with surgeon about 1-α-calcitriol only in BNE or re-op, Review ~ day 5
  - **PTH ≥ 1.6**
    - **Ca\(^{2+} < 2.2****: Oral calcium 1g tds, Review ~ day 5
    - **Ca\(^{2+} ≥ 2.2****: No treatment required (consider oral calcium for symptoms, borderline levels, or living far from hospital, but not in those with renal impairment or stones)