A prospective, controlled study on 131 patients assessing patient safety and nasal function outcomes following human olfactory mucosa biopsy as a source of cells for central nervous system regeneration during endoscopic sinus surgery

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DECLARATION

‘I Peter Andrews confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.’
ACKNOWLEDGEMENTS

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ABSTRACT

Title
A prospective, controlled study on 131 patients assessing patient safety and nasal function outcomes following human olfactory mucosa biopsy as a source of cells for central nervous system regeneration during Endoscopic Sinus Surgery (ESS).

Hypotheses
The primary hypothesis states; olfactory harvesting is a safe procedure and does not incur a reduction in nasal function including the sense of smell when compared to a control group. The secondary hypothesis states; ESS improves olfactory outcome in CRS patients with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP).

Materials and Methods
Full Ethical and Research and Development (R&D) approval was granted; Ref: 05/Q0512/103. 131 patients were recruited over a 2 year period and non-randomised into the olfactory biopsy and control arms. Statistical significance was accepted at the 5% level (<0.05) and powered at 80%. Complication rates as well as patient and surgeon reported outcome measures were recorded in each arm both pre operatively and 6 months post operatively. The sense of smell was evaluated using the University of Pennsylvania Smell Identification Test (UPSIT).

Results
65 patients underwent superior turbinate biopsy with 66 controls. The complication rate, the nasal function and the sense of smell outcomes of the biopsy group were statistically the same when compared to the control group. In the CRS subgroup analysis the sense of smell improved in both groups following ESS but only in the CRSwNP subgroup was it found to be significant.

Conclusions
The primary hypothesis was shown to be true and demonstrated that patient morbidity and beneficial outcomes following harvesting human olfactory nasal mucosa during ESS is statistically the same when compared to the control group. The secondary hypothesis was equally shown to be true and demonstrated that sinus surgery improved olfaction in both the CRSwNP and CRSsNP subgroups but only in the CRSwNP subgroup was the olfactory improvement significant.
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SYMBOLS AND ABBREVIATIONS

FESS  Functional endoscopic sinus surgery
ESS   Endoscopic sinus surgery
QOL   Quality of Life
PROM  Patient reported outcome measure
OEC   Olfactory ensheathing cell
CNS   Central Nervous System
SNOT 22 Sino-Nasal Outcome Test 22
SNOT 23 Sino-Nasal Outcome Test 23
VAS   Visual Analogue Scale
NOSE score Nasal Obstruction Symptom Evaluation
UPSIT University of Pennsylvania Smell Identification Test
LK score Lund Kennedy score
LM score Lund Mackay score
CRS   Chronic Rhinosinusitis
CRSwNP CRS with Nasal Polyps
CRSsNP CRS sans Nasal Polyps
EP\textsuperscript{3}OS European Position Paper on Rhinosinusitis and Nasal Polyps
CSF   Cerebrospinal Fluid
ORN   Olfactory Receptor Neurons
PNS   Peripheral Nervous System
GBS   Globose Basal Cells
HBS   Horizontal Basal Cells
CT    Computerised Tomography
MRI   Magnetic Resonance Imaging
OB    Olfactory Bulb
OMC   Osteomeatal complex
AON   Anterior Olfactory Nucleus
NIPF  Nasal Inspiratory Peak Flow

Keywords: Smell, Nasal Surgery, Rhinitis, Olfactory Mucosa, Glia, Quality of Life
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CHAPTER 1

INTRODUCTION

The unmet need

Nasal olfactory mucosa is an accessible source of Olfactory Ensheathing Cells (OECs) which can be harvested for spinal cord and brachial plexus repair. Current clinical studies utilizing OECs in central nervous system (CNS) regeneration have shown promising results (1-3). In order to move into clinical trials of OECs for nerve repair there is a need to perform patient safe olfactory mucosa harvesting (4). Current evidence suggests that human olfactory mucosal biopsies do not have a detrimental effect on the patient’s sense of smell (5,6), however up to now a prospective controlled study measuring olfaction, quality of life as well as patient and surgeon reported outcome measurements has not been performed. In Feron et al, 20 patients had undergone olfactory biopsies and their sense of smell also had not deteriorated as measured by the UPSIT measurement (6). In Lanza et al, a retrospective olfactory study was performed which evaluated 19 patients who had undergone olfactory biopsies. According to their UPSIT findings a reduction in the sense of smell, was not demonstrated (5). Equally the surgical techniques for nasal olfactory harvesting which have been reported in the literature have recruited small numbers of patients as well as lacking robust safety, nasal function and quality of life analysis (7,8).

Background/Introduction

OEC yield rates from nasal septum mucosa, which is more accessible than superior turbinate mucosa, during routine endoscopic nasal surgery has been explored but unfortunately yield rates were low which has spearheaded this thesis (9). Endonasal techniques for optimizing nasal olfactory harvesting have been developed so as to maximize OEC yield rates and current evidence suggests that the superior turbinate is both accessible and also allows for an optimal OEC yield (4). Anatomically human autopsy techniques have demonstrated maximal olfactory tissue to be present beneath the cribiform fossa including the superior septum however such techniques would prove difficult to replicate during routine nasal surgery owing to the likely hood of cerebrospinal fluid (CSF) leakage (10). Consequently the superior turbinate harvesting technique has evolved which utilises the middle section of the superior
turbinate. The superior turbinate lies close to the cribiform niche and the intrinsic olfactory tissue and will be further described in this thesis (4).

The quest to repair the human central nervous system (CNS) following trauma remains a surgical challenge. The human olfactory system may provide an answer by utilizing the regenerative ability of the olfactory ensheathing cell (OEC), which facilitates the natural neurogenesis of the human olfactory nerve during the olfactory life cycle and uniquely is the only glial cell to facilitate regeneration within the CNS. The concept of utilising OECs for CNS repair is predicated on their intrinsic ability to facilitate olfactory neurogenesis and supports regenerating axons within the CNS. OECs could be harvested from the olfactory bulb however this would be associated with unacceptable risk of stroke, seizure or death (11-13), hence a safer alternative is being advocated.

Olfactory ensheathing cells (OECs) are glial cells which specifically support the olfactory receptor neurons (ORNs) and are located both in the olfactory bulb of the CNS and the olfactory mucosa of the peripheral nervous system (PNS) (14-16). They were described by Doucette in 1984 as a distinct glial cell entity enabling regeneration of the olfactory system (17). They support, nurture and facilitate regeneration of the olfactory nerves and have the unique ability to transgress both PNS and CNS environments (18). They also provide important neuro-protective properties and stimulate regeneration through neurotrophic signaling and myelination of new axons, providing a continuous channel for the regeneration of new olfactory axons (12). OECs have the unique ability to interact and migrate within the astrocytic rich environment of the CNS and human olfactory bulb (19) and have been shown to remyelinate de-myelinated CNS axons (15;18).

**Spinal cord injury and Glial Scar Formation**

The inability for the mammalian CNS to repair itself following injury is thought to be an evolutionary safety mechanism so as to prevent catastrophic disorganized re-growth. Although, the mammalian brain after injury does exhibit plasticity with the ability to form new synapses, unfortunately it is unable to form new axons (20;20). This inhibitory nature of the injured central nervous system is predominately a consequence of the formation of a glial scar which contains inhibitory proteins and prevents axonal re-growth (21).
Conversely, the human olfactory system undergoes a regular cycle of neurogenesis whereby olfactory neurons have the unique ability to regenerate into the inhibitory CNS environment on a regular basis and is facilitated by olfactory ensheathing cells (OECs). This extraordinary process offers a potential remedy for CNS regeneration by laying down a non-inhibitory pathway and by facilitating regeneration allows neurons to regenerate in the CNS \(^{(18)}\).

**Glial scar formation**

Glial scar formation occurs as a result of CNS injury through a process of astrocytosis. Through the aggregation of astrocytes inhibitory proteins are laid down and include nogo amyelin associated glycoprotein and oligodendrocyte myelin glycoprotein \(^{(21-24)}\). Astrocytes represent one of the four CNS glial supporting cells which also include oligodendrocytes, microglia and olfactory ensheathing cells (OECs). The role of the astrocyte is one of protection as well as in the formation of the blood brain barrier. Oligodendrocytes equally provide a supportive neuronal role as do microglia within the CNS. OECs are the supportive glial cell for the olfactory receptor neurons (ORNs) \(^{(14,23)}\).

**Transplantation technology**

The ever increasing need to surgically repair the CNS has fuelled the need to harness techniques which encourage regeneration and work against the intrinsic inhibitory nature of the human CNS. The transplantation of regenerative cells into the injured CNS site is one potential solution which has been explored in the injured peripheral nervous system as well as being harnessed in treating diseases which require replacement therapy such as Parkinson’s disease \(^{(25,26)}\). The peripheral nervous system (PNS) has an inherent ability for regeneration unlike the central nervous system. The process of regeneration in the peripheral nervous system is multi-factorial and includes the ability for the regenerating neuronal end plate to penetrate and re-grow through the surrounding substrate of a Schwann cell conduit. Importantly PNS regeneration is not met with an inhibitory glial scar equivalent \(^{(27,28)}\).

The concept of utilizing olfactory ensheathing cells as a source for transplantation is predicated on its ability to regenerate into the inhibitory arena of the central nervous system and the added advantage of being autologous tissue. The alternative to an autologous
transplantation of olfactory tissue would be the transplantation of allogeneic olfactory cells but this would require immunosuppression on behalf of the recipient. The key however is devising a safe and reproducible olfactory harvesting technique. The process of harvesting OECs which were originally sourced from the olfactory bulb in the rat obviously would pose significant safety issues in the human albeit a potential calculated risk in a paraplegic patient with a burning quest to walk again\(^ {11;13}\). In the human an alternative safer technique involves harvesting OECs from the nasal mucosa. Methods of nasal olfactory harvesting which significantly reduce the risk of morbidity have been devised and used in autologous transplantations in paraplegic patients\(^ {2;3;7;8}\).

The concept of cellular transplantation of human OECs into an injured spinal cord lesion is being developed and trialed\(^ {1}\). There have been mixed results owing mainly to the lack of uniformity and consistency between the different regenerative units pertaining to their grafting techniques and protocols. This has lead to difficulty in ascertaining the ideal and optimum constituents for OEC grafts. Although it would make sense for a pure OEC graft transplantation to be the gold standard, current strategies favour a less pure constituent which also contains fibroblasts; based on the possible hypothesis that the 2 cell types are synergistic. The estimated number of pure OECs required for human spinal cord transplantation is unknown and is obviously dependent on the size of the lesion although it is thought to be between the region of \(10^7\) and \(10^8\)\(^ {3}\). The reality is that the exact regenerative component of the olfactory epithelium is also unknown and may additionally require its basal cell constituent\(^ {26}\). The method of transplantation of OECs into the patient does vary within the literature, and depends on the size of the defect, and includes injecting expanded cultures of OECs via micromanipulation whereas in other studies the whole of the olfactory mucosa is inserted\(^ {23}\). The advances in tissue engineering and scaffold implantation technology will revolutionise the future paradigms of spinal cord injury treatments\(^ {29}\).

**Origin of OECs**

OEC’s show both Schwann cell and Astrocytic cell immunocytochemical properties owing to their common glial identity and embryonic origin. OECs are embryonically derived from the neural crest as are Schwann cells although until recently they were thought to be derived from the olfactory placode\(^ {30}\). OECs and Schwann cells both exhibit expression for the neurotrophin receptor; the low affinity nerve growth factor receptor p75 NTR\(^ {31}\), the
polyclonal antiglial fibrillary acidic protein anti-GFAP and S100 beta. As a result both OECs and Schwann cells stain positive to these antibodies which makes differentiation challenging in vitro and the ability to rule out Schwann cell contamination difficult (23). Recent work has shown OECs express smooth muscle actin (SMA) whereas Schwann cells do not express it (32) and equally the anti-HNK1 antibody is a specific antibody for Schwann cells which has been used to determine whether there is evidence of significant trigeminal Schwann cell contamination within in-vitro cultures of human nasal OECs (7).

OECs in the olfactory bulb are predominately the same cell type as OECs found in the lamina propria of the olfactory mucosa, although subtle differences have been demonstrated. OECs residing in the olfactory mucosa possess more migratory properties (33) and potentially more stem cell characteristics (34). It was initially thought that because rats being macrosmatic (i.e. 50% of their nasal cavity surface area is dedicated to olfaction) they contained a different cell type of OEC to microsmatic humans. However, transplantation of human OECs into an immunosuppressed severed rat spinal cord showed integration and remyelination and hence demonstrated an equivalent universal cell type (26;35).

**Human Olfactory Receptor Neuron (ORN) and Cribiform plate anatomy**

The human olfactory receptor neurons (ORNs) regenerate and replenish themselves every 4 to 6 weeks through a process of neurogenesis whereby new axons travel back through the cribiform foramina of the cribiform fossa and pass into the astrocytic rich CNS environment and re-synapse within the olfactory bulb. The ability of OECs to support regenerating axons within the CNS has lead to their use in transplantation technology (36;37). Olfactory receptor neurons are bipolar, ciliated neurons. The proximal end is non-myelinated and then forms myelinated bundles of axons called filia olfactoria as they congregate in the lamina propria. In the lamina propria the olfactory nerve bundles are immunoreactive to beta tubulin and neurofilament. The distal end forms knob like structures which give off cilia-like extensions containing receptors, each neuron gives off up to 50 extensions which sit in the mucous layer (16). The extensions contain the olfactory receptors for which there are approximately 1000 different types, and each respond to a different type of olfactant. Axel and Buck demonstrated that each ORN expressed only one olfactory receptor protein and belong to the family of g protein coupled receptors (38). They also estimated that there were 1000 different genes for the olfactory receptors in the human genome. All neurons expressing the same receptor synapse.
on the same olfactory bulb glomerulus.

The proximal olfactory neurons join together and form bundles of axons which then pass through the cribiform plate. There are approximately 20 bundles in each cavity. Each olfactory bundle pierces through the cribiform plate perforations of the ethmoid bone with each measuring less than 1mm in diameter and synapse within the olfactory bulb. As the nerves exits through the cribiform fossa they divide into 2 groups; the inner and outer groups. The inner group being the larger is distributed along the perpendicular plate and the outer group supplies the lateral nasal wall and superior turbinates. The cribiform plate of the ethmoid bone is narrow and deeply grooved. It supports the olfactory bulb which continues on as the olfactory tract. The cribiform plate consists of two to three rows of perforations allowing the olfactory nerves to penetrate. The inner row contains larger perforations but fewer in number and allows the passage of the ONs from the septum/perpendicular plate. The perpendicular plate is grooved to allow the passage of the ONs. The middle and outer rows contain the smallest diameter perforations and supply the roof and the superior turbinates\(^{(39)}\).

**Figure 1**

Olfactory nerve bundles piercing the cribiform fossa from the nasal septum

ONB=Olfactory Nerve Bundles
Stem cell regenerative properties of the olfactory system

The human olfactory system undergoes neurogenesis every six weeks in order to replenish ORNs which are exposed to harmful and toxic substances resulting in injury and death and this process continues until the age of 72 years \(^{(40)}\). \(\text{Olfactory neurogenesis} \) is a combination of regeneration and programmed apoptosis. ORNs are replenished from basal cells which are putative stem cells found in the olfactory epithelium. Stem cells by definition represent an undifferentiated cell within a multicellular organism which are capable of giving rise to indefinitely more cells of the same type and from which certain other kinds of cell arise by differentiation \(^{(18)}\). The other 2 areas within the CNS which are known to undergo neurogenesis include the olfactory bulb and hippocampus \(^{(16)}\). However, despite the intrinsic ability of neurogenesis within the olfactory system it still remains frustrating that the olfactory system itself remains prone to irreversible damage.

The specific cell types within the olfactory epithelium which are involved with the process of olfactory neurogenesis include ORNs, OECs and Basal cells. Basal cells act as putative stem cells and there are 2 types in the rat; Globose Basal Cells (GBCs) and Horizontal Basal Cells (HBCs). The GBCs sit above the HBCs on the epithelial basal lamina superficial to the lamina propria. The GBCs are rounder and are involved in the normal replenishment process of the ORNs and OECs as well as the sustentacular supporting cells. It is thought the HBCs are quiescent but come into play during extreme injury and have the ability to replenish the GBCs as well as the ORNs/Glial cells. Hence HBCs are thought to be the true stem cell population \(^{(18)}\). In the human however there appears to be only one type of basal cell which morphologically resembles more the rhodent GBC, as opposed to the rodent HBC, and the reason why humans only have one basal cell type remains unclear \(^{(42)}\).

The olfactory nerve bundles within the lamina propria are also enveloped by fibroblasts and consequently immunostain for beta tubulin and neurofilament. Interestingly it’s difficult to determine which combination of cells within the olfactory epithelium are ideal for transplantation however studies have demonstrated that pure isolates are not ideal and instead a combination of OECs and fibroblasts exhibit a more favorable regenerative ability. Animal studies have shown regeneration is only effective if OECs and fibroblasts are transplanted together which is not surprising given fibroblasts play a vital role in connective tissue production and supporting OEC function and given the ‘feeder cell’ relationship in vitro \(^{(12)}\).
The aim however is to ensure the proportions of OECs and fibroblasts are optimal. Fibroblasts have been shown to proliferate in the first week of cell culture, OECs in the second week and then over proliferation of fibroblasts in the third week. There are methods to amplify OEC cultures which include suppression of fibroblast proliferation using cytarabine and antibody-mediated complement killing, separate the cells using fluorescent-automated cell sorters, implementation of growth factors to encourage the growth of OECs and exploring the use of allogenic OEC cell lines (4;9;23).

**Harvesting Olfactory Mucosa**

The keys to harvesting human olfactory mucosa are to ensure the technique is ‘patient safe’ and yet ensures a maximum yield rate of OECs. Technically, the ideal place to harvest human OECs is from the areas of highest olfactory receptor and axonal density. This corresponds to the area where the distal olfactory bundles exit the nasal cavity near the cribiform plate, superior septum and superior turbinate (10). The surgical challenge is to ensure high yield rates of OECs which reside superiorly but without breaching the anterior skull base. If the anterior skull base is breached with a resultant cerebrospinal fluid leak, then the patient is exposed to the potential risks of meningitis and cerebral damage. Safe techniques have been described in the literature with regards to minimising anterior skull base trauma and reducing post operative morbidity with regards to loss of smell (43). Lanza et al also concluded several punch biopsies can be taken from the olfactory area without causing detriment to the sense of smell, albeit the study contained small numbers and hence it was difficult to draw absolute conclusions (5).

However, surgical harvesting techniques optimizing yield rate of OECs as opposed to olfactory mucosa have only been robustly evaluated by Choi et al (4). Bianco et al had assessed the OEC yield rate from 3 superior turbinate biopsies but without significant conclusions (7). OEC yield rates are higher when harvested from the superior turbinate region as opposed to the septal area and are equally higher from younger and more sinonasal disease free mucosa with OEC yield rates averaging 25% (4). It must be noted that embryonically the olfactory mucosa within the nasal cavity is a continuum but over time and with degeneration secondary to age and disease, it is replaced with respiratory epithelium (44). The limitations of OEC harvesting from nasal septal mucosa have been demonstrated particularly in the caudal
regions (9). Interestingly, Choi et al described their optimum OEC yield rates based on the calculated percentage covering of a 35mm plate whereby yield rates of more than 50% covering were considered large enough to proceed for the treatment of patients (9). The optimum 50% OEC yield rate is based on earlier rat experimentation models whereby similar 50% yield rates would contain approximately 1.5 million OECs. In their rat spinal cord lesion model approximately 500,000 OECs and 500,000 fibroblasts would be injected into the lesion affording improved functional recovery (45). The human model is based on similar concepts but a multiple of 100 as based on Choi et al unpublished data.

**Sinonasal Anatomy**

The anatomy of the paranasal sinuses is generally well defined and consists of five pairs of sinuses; the anterior and posterior ethmoidal, maxillary, frontal and sphenoidal sinuses. However, there is variability in the height of the skull base and pneumatization of the sinuses which necessitates the need for preoperative CT scanning in order to delineate this variation prior to FESS (46). The skull base does not slope back posteriorly at the same height but instead slopes back inferiorly from the frontal sinus ostium to the sphenoid as shown in figure 2, which is highlighted in the sagittal CT image guidance scan. In figure 2 the height of the skull base reduces from the frontal sinus anteriorly to the sphenoid sinus posteriorly over a distance of 3 cms. The anterior skull base also represents an area of increased weakness particularly the lateral lamella of the ethmoid bone (46).
Figure 2 Image Guidance CT scan with saggital image delineating the sloping anterior skull base
Figure 3

Coronal CT scan highlighting the asymmetry of the anterior skull base with a significant deviation of the septum to the left and a left sided concha bullosa

Olfaction

Olfaction or the sense of smell is the detection of odorants and represents the oldest human sense. It is one of Aristotle’s five senses; the other four being taste, vision, sight and touch. The olfactory system’s limbic and higher cortical connections play an important role in motivation and reward which can stimulate intense, enduring odour memory \(^{(47;48)}\). Consequently, in cases of olfactory dysfunction there can be a reduction in quality of life, with resultant depression, weight disturbance, social anxiety and social isolation \(^{(49)}\). Human olfaction as well as providing sensual pleasure through the recognition of perfumes and memory association also aids in survival, with warning of danger by detection of rotten
smells and smokes. There are specific professions which are dependent upon an acute sense of smell including chefs and sommeliers/tea tasters. There are tens of thousands of odorants and the human nose has the ability to discriminate from 1 trillion odours. However in other animals its role is more substantial and acts as a pre requisite for survival facilitating the hunting of food, mating, avoiding danger and maternal care \(^{(50)}\).

The nose has 2 main sensory inputs; the detection of odorants and the perception of nasal airflow. The primary function of the nose is to optimize the quality of inspired air and in doing so act as an ‘air conditioner device’ enabling the filtration, warming and humidification of inspired air. The nose shares its’ air conditioning function with the olfactory system which consists of 2 organs, each innervating one nasal cavity. Unlike vision and hearing the nose shares its sensory function with other functions of the nose. Olfaction requires a fluid environment in order to allow the odorants to dissolve and also requires a non-obstructed and patent airway \(^{(51)}\).

**Olfactory physiology and location of olfactory mucosa**

There are approximately 10,000,000 olfactory receptor neurons supplying the human olfactory mucosa although this is thought to be an under estimation. The total surface area of the olfactory epithelium is 2.5cm squared and equates to the size of a postage stamp with 1.25cm squared in each nasal cavity \(^{(52)}\). The total surface area of the internal mucosal nasal cavity is approximately 50cm squared and the olfactory mucosa accounts for 3% in the human compared to 50% in the rat. In the dog there are approximately 4 billion olfactory receptor cells. The olfactory receptor area lies in the dorsal superior aspect of the nose with close proximity to the cribiform plate and anterior skull base and equates to the superior turbinate and dorsal septum \(^{(52)}\).

The exact location of the olfactory mucosa within the nasal cavity remains ill-defined and generally shown to be more concentrated in the dorsal aspect of the nose including the posterior, superior septum and superior turbinate \(^{(43;53)}\). Feron et al, studied 33 healthy subjects and found yield rates were higher when biopsies were taken from the superior turbinate \(^{(43)}\). Leopold interestingly studied healthy volunteers and found olfactory tissue more anteriorly than originally thought which correlated to positive electro olfactograms (EOGs) \(^{(44)}\). It would
potentially equally explain why patients could still smell even when their olfactory niche was blocked secondary to sinonasal disease. As well as objective electro-olfactograms (EOGs), patients also underwent olfactory biopsies which were immunostained for olfactory marker protein (OMP) and beta tubulin 3 which is a neurofilament antibody specific for neurons. Over 50% stained positive, with some samples only staining positive in the lamina propria and not the epithelium signifying olfactory degeneration (44). Lane et al studied the inferior aspect of the superior turbinate and found olfactory neuroepithelium (53). Choi et al, evaluated septal mucosa and although olfactory mucosa was successfully harvested the olfactory yield rates were deemed too low for potential transplantation as previously described (9).

**Odorants and Olfactory Receptor Genetics**

The initial step in olfactory transduction is the movement of odorants from the air phase of the nasal cavity into the aqueous phase of the olfactory mucus. Odorants are carried into the nasal cavity and through nasal airflow turbulence are distributed superiorly towards the olfactory niche. They subsequently dissolve in the mucous layer and attach to a specific odorant binding protein. In order for an odor to be perceived ideally it must possess molecules with water solubility, high vapor pressure, low polarity and lipophilic activity. Most odorants are hydrophobic and not water soluble and hence require odor binding proteins. Odors are then coded by specific patterns of ORN action potential neural activity either in space or time which leads to the perception of odors (54). Although the current concept of odorant recognition is through shape identification there is increasing evidence also supporting molecular vibration (55).

Buck and Axel described a marked genetic diversity in olfactory receptors and found in rodents a large multigene family of ~1000 genes which appear to code for odorant receptor proteins with seven trans-membrane domains. However, in humans, more than half of the receptor gene families are pseudogenes and through epigenetics and the process of turning genes on and off there are approximately 500 functional receptors. Even though each receptor cell expresses only one type of olfactory receptor, such cells respond to a wide range of odorants. However, a given receptor, though a “generalist,” does not respond to all stimuli to which another receptor responds, thereby allowing for cross-neuron quality coding (38).
The olfactory and respiratory epithelium

The olfactory epithelium consists of non ciliated pseudocolumnar epithelium and to the naked eye looks identical in colour to the surrounding respiratory epithelium, unlike the rodent which exhibits a yellow hue (56). The cellular composition of the olfactory epithelium consists of four types of cell; ORN’s, supporting glial cells; OECs and basal cells. The basal cells represent the putative stem cells and are divided into small globose basal cells and horizontal basal cells which lie on the basal lamina (18). The olfactory epithelium in the human is a lot thinner compared to the rodent whereas the lamina propria is a lot thicker (9).

Respiratory epithelium is a ciliated pseudostratified epithelium and each cell contains approximately 250 cilia which are 2 to 5 microns in length. Each cilia consists of 9 microtubules doublets surrounding 2 microtubules and held together by dynein arms. Cilia beat with a coordinated biphasic wavelike rhythm called metachronism, 1000 strokes per minute with a powered forward stroke and a slow recovery stroke. They transport particles in the nose at 20 micrometers per minute and can be objectively investigated using the saccharine test with a normal time of 20 minutes (51).

Surgical anatomy of the first olfactory nerve

The first olfactory nerve represents the most caudal olfactory nerve bundle which is often the first olfactory nerve bundle identified during surgical dissection piercing the cribiform plate, just anterior the anterior ethmoidal nerve. It lies adjacent to the perpendicular plate and septum and is often seen during endoscopic frontal sinus surgery and exposed whilst locating the olfactory protuberance during an endoscopic modified Lothrop procedure, as shown in figure 4. It represents the anatomical landmark for the olfactory protuberance and hence the anterior skull base (57).
Figure 4
The first Olfactory nerve exiting through the cribiform fossa, identified under the suction tip

Trigeminal innervations

Although olfaction is primarily detected through the first cranial nerve, the fifth, ninth and tenth cranial nerves also provide olfactory input. The trigeminal nerve is thought to play a role in the modulation of olfactory information, as well as recognition of pungent smells. It provides sensation to the inside and outside of the nose as well as perception of nasal airflow through its somatosensory innervations (58). The trigeminal sensory receptors are distributed anteriorly in the nares as opposed to the more superiorly localized olfactory receptors in the attic of the nasal cavity. Different afferent fibres are involved in the trigeminal mediated sensation; ‘C fibres’ transmit dull, burning pain and ‘A fibres’ transmit sharp stinging pain.
The first and second divisions of the trigeminal nerve are involved in the sensory provision of the nose and are involved in the initiation of the protective feedback mechanism including sneezing. The majority of odorants stimulate both the olfactory and trigeminal receptors such as nicotine providing concurrent odor perception and stinging. However, the odorant Vanillin only stimulates the olfactory receptors \(^{(58)}\). The trigeminal anterior ethmoidal nerve is one of the first nerve bundles encountered during anterior skull base exploration and is several millimeters anterior to the first olfactory nerve.

**The vomeronasal organ**

The vomeronasal organ (VNO), or organ of Jacobson, is an accessory concentration of olfactory tissue. It is located in a 1 mm to 3 mm tubule, with an oval orifice, approximately 1 cm posterior from the caudal septum and 2 to 4 mm off the floor of the nose. It is believed to be receptive to pheromones which are responsible for sexual instincts and mood. VNO is present in 91% of the population and thought to be a rudimentary organ without neuronal connection to the brain \(^{(59)}\).

**The Olfactory bulb**

The human olfactory bulb (OB) is an elongated evagination of the forebrain which lies on the cribiform niche and receives 20 olfactory bundles from each nares. Its function is thought to act as a filter of olfactory inputs. Its average volume is 125 mm\(^3\) and shown to be smaller in females. The volume of the OB remains ‘plastic’ and varies in size according to its neuronal stimulation owing to its intrinsic ability to undergo neurogenesis. It has been shown to be smaller in patients with olfactory disorders and equally smaller on the same side of a deviated septum with subsequent reduced olfactory stimulation \(^{(39,60)}\).

The OB represents a highly organized structure which consists of 5 layers; the outer glomerular layer, external plexiform layer, mitral cell layer, internal plexiform layer and the granule layer. The outer glomerular layer is formed from dendritic tangles of mitral cells which synapse with excitatory ORNs as well as inhibitory periglomerular and granule cells. The olfactory bulb contains on average 5500 glomeruli \(^{(61)}\). ORNs with the same receptors diverge onto the same glomerulus. Approximately 10,000,000 ORNs synapse with 5000
glomeruli. The mitral cell axonal projections run caudally through the OB structure and onto the olfactory cortex\(^{(61)}\).

Figure 5
T2 weighted MRI scan highlighting T2 hyperintense olfactory bulbs on the cribiform fossa

The olfactory bulbs can be seen as evaginations of the anterior cerebral cortex in the above T2 weighted MRI scans. In figure 5 the olfactory bulbs are T2 hyperintense and lie on the
cribiform fossa and in figure 6 the extension of the olfactory bulb and its olfactory neurons can be seen coalescing onto the superior turbinate.

**Figure 6**

T2 MRI scan highlighting hyperintense olfactory bulbs coalescing into the superior turbinate

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**Olfactory Cortex**

The olfactory lobe or cortex (brodman area 34) in the human is a rudimentary elongated structure and lies beneath the frontal lobe and is bordered laterally by the temporal lobe and indents the underlying frontal lobe by the formation of a sulcus (39).
The Mitral cell axons project to the olfactory cortex via the olfactory tract. The anterior olfactory nucleus (AON) is the first structure to receive projections from the olfactory bulb via the lateral olfactory tract. The AON plays an important role in the perception of odorants and is often thought to represent the anterior olfactory cortex which then relays connections onto the primary olfactory cortex in the medial temporal lobe and the secondary olfactory cortex in the orbitofrontal cortex, with some fibres crossing over to the contralateral side (39).

The primary olfactory cortex includes the piriform cortex as well as the olfactory tract, the uncus of the hippocampus, and the anterior part of the parahippocampal gyrus. The entorhinal area or secondary olfactory cortex connects to the thalamus, basal forebrain and the limbic system (39). The priform cortex is involved with odour recognition and the entorhinal area more associated with memory and smell associations. The thalamus is thought to be involved in the conscious level of smell perception whereas the amygdale and entorhinal areas represent the limbic system and the subconscious perception. The olfactory system is the only sensory system that has direct cortical projections without a thalamic relay nucleus (39,62).

**Perception of Nasal Airflow**

One of the five functions of the nose is nasal breathing, as well as olfaction, humidification, filtration and warming. The perception of nasal breathing or airflow is mediated and controlled through the somatosensory afferents of the trigeminal nerve. This feedback process ensures optimal nasal patency, although can remain disordered even in the presence of a patent airway. The majority of the inspired nasal airflow runs inferiorly along the nasal cavity and the percentage of airflow directed to the olfactory region with each breath under resting conditions is about 10% (63). Sniffing increases the percentage of inspired air at the olfactory area to 20% (250 ml) making the perception of smell more appreciable (64).

The complaint of nasal airflow blockage correlates poorly with objective findings and remains a challenge to rhinologists (65). Patients with large, typically post operative nasal cavities still perceive the sensation of nasal blockage (64). Nasal airflow during normal nasal
breathing passes through the external nasal valve in a laminar flow pattern and its velocity is approximately 2-3 m/s. The air then passes through the internal nasal valve, the narrowest part of the nasal cavity, and speeds up significantly to 12-18 m/s. The air stream then becomes more turbulent as it then flows into the nasal vault owing to the larger cross sectional area and its speed reduces to 2-4 m/s. Turbulence is a precondition for gaseous interaction between the air and mucosa which is vital for warming, humidification, filtration and olfaction. The middle turbinate then acts as an air foil enabling a change in direction of airflow and splits the airstream so as to waft superiorly into the olfactory cleft.

The nasal mucosa as well as being lined by respiratory mucosa also consists of a vast network of erectile vascular tissue containing arterioles, arteriovenous anastomosis and venous sinusoids which enables the controlled process of engorgement. The process of congestion is under humerol and sensorineural control and ensures optimal resistance and the ideal turbulent airflow. The nasal cycle is a physiological phenomenon whereby the left and right nasal cavities alternate in nasal congestion whereby the resistance is increased on one side and decreased on the other. The “intrinsic nasal cycle” is present in about 80% normal individuals, wherein only one nostril remains fully patent at one time. It usually lasts 2-6 hours and alternates between the nostrils. The overall resistance remains the same. This process was first described by Kayser in 1895. Sweat and lacrimal secretions are involved in chemosignalling which incur both behavioural and hormonal change in others; including menstrual synchrony and the smell of both fear and empathy.

An odorant may reach the olfactory mucosa through 2 different routes; sniffing through the nose (orthonasal) and via the mouth (retronasal). The latter process facilitates the flavours of foods and has been shown through functional MRI to be processed differently within the olfactory cortex. The nose provides 2 different olfactory inputs to the brain one from each nostril and although not truly understood this dual process is thought to play a role in localization of smells with the ability to find food.

Objective Measurement of olfactory activity

An electro-olfactogram (EOG) measures the action potential activity of olfactory receptor neurons (ORNs) by electrode placement on the olfactory mucosa. However, olfactory
event related potentials are now universally used which are measured via electrode placement on the scalp following an odorant stimulation. The olfactory event-related potentials (OERPS) are specific electroencephalography (EEG) recordings triggered by an odorant stimulant. The odorant is introduced via an olfactometer which enables repeatable and calibrated odorant delivery. In anosmic patients they are absent and in hyposmic patients they are present in approximately one third of patients and therefore require additional psychophysical olfactory measurements including Sniffin sticks or UPSIT tests (68). OERPs play a role in predicting olfactory recovery following a viral infection and if found early in the recovery process convey a positive predictor for a favourable outcome (69). Event related functional MRI equally measures objectively olfaction by mapping out cerebral activity following an olfactory stimulant and has been used in determining olfactory cerebral pathways (70).

**Objective nasal airflow measurements**

Rhinomanometry is the objective measurement of nasal resistance and is calculated by measuring air flow and pressure within the nose and extrapolating the respective resistance. The internal nasal valve area can be measured using acoustic rhinometry which measures the cross sectional area of the nasal cavity as a function of longitudinal distance along the floor of the nasal cavity. The nasal cavity is divided into anterior, middle and posterior segments. The anterior segment which is the first 3 cms of the nasal cavity corresponds to the nasal vestibule and internal nasal valve and contains only very limited congestive capacity otherwise known as the minimal cross sectional area 1 (MCA 1) whereas the middle third which measures from 3cms to 5.2cms or MCA 2 contains the inferior and middle turbinate areas and represents the main congestive area (51).

**Olfactory dysfunction**

**Definition of Anosmia**

Anosmia is the inability to smell, hyposmia is the reduced ability to smell and normosmia is the normal ability to smell. Parosmia is the distorted ability to smell and Phantosmia is the ability to smell in the absence of an odorant. Physiological anosmia occurs as a result of odorant saturation during prolonged smelling of a high intensity odorant such as vanillin for
more than 15 minutes. The ORN binding receptors become saturated and this phenomenon is reversible \(^{(48)}\).

**Aetiology**

Olfactory dysfunction is frequent and can profoundly influence a patient’s quality of life. Olfactory dysfunction has been reported to affect approximately 5% of the UK population with a prevalence of over 25% in other published population studies \(^{(49)}\). As a sensory disorder it is more common than blindness and deafness and carries significant psychosocial consequences for the sufferer \(^{(71;72)}\). Hyposmia is present in 16% of the population and rates are significantly higher in patients with rhinological disease \(^{(73)}\). It rarely presents itself in isolation or in the absence of trauma, but usually with other symptoms of nasal pathology including nasal obstruction and rhinorrhoea.

Anosmia is a recognized symptom which helps qualify the diagnosis of chronic rhinosinusitis according to the EPOS guidelines \(^{(74)}\). Studies have also shown that olfactory dysfunction affects over 50% of the population older than 65 years \(^{(75)}\). Philpott et al, demonstrated that 55% of patients who complained of hyposmia had low combined olfactory test (COT) scores whereas 33% of patients with a low COT score had no subjective hyposmia \(^{(76)}\).

**Pathogenesis of Anosmia**

The commonest causes for anosmia are head injury, viral upper respiratory tract infection and CRS and they can account for up to 80% of presentations \(^{(49;77)}\). Olfactory disorders can be classified as conductive, sensory or neural disorders \(^{(78)}\). Conductive disorders reflect diminished access of odorants to the olfactory neuroepithelium. Sensory disorders on the other hand, involve direct damage to the neuroepithelium. Sensory disorders reflect injury to the olfactory bulb and central olfactory pathways.

**Treatment of Anosmia**

Hyposmia caused by CRS is thought to be the most amenable to therapeutic interventions. Most hyposmic patients are thought to suffer with allergic rhinitis, chronic rhinosinusitis or nasal polyposis. The mainstay of treatment for this group is intranasal/topical corticosteroids \(^{(79-81)}\). Bugten et al, showed that patients with CRSwNP complain more of nasal blockage and a reduced sense of smell whereas patients with CRSsNP complain more of facial pain and headaches, interestingly they did not find a significant difference in subjective symptom
improvement, with both groups responding similarly following FESS $^{(82)}$.

**The role of FESS in CRS**

The role of FESS in sinonasal disease is well documented and according the EPOS guidelines is indicated when medical treatment fails. Its main objective is to reduce nasal obstruction and to enable ease of passage of topical steroids into the sinonasal openings. The evidence supporting the efficacy of FESS in the treatment of CRS with or without polyps shows it to be as effective as medical treatment, but not better $^{(74,83)}$.

**Medical Treatment**

Studies have shown that steroids either in the form of a nasal spray or drops are effective in treating hyposmic patients with concurrent nasal disease. Golding-Wood et al showed that using topical steroids (bethamethasone) daily for six weeks improved the UPSIT test scores significantly in their small hyposmic cohort and suggesting that topical steroid therapy is effective in treating perennial rhinitis and nasal polyps who also suffer with hyposmia $^{(84)}$. A study by Chalton et al, found that the distribution of the steroidal drops are enhanced when taken in the 'head down' or Moffats position and is believed to encourage maximum exposure of the drug to the nasal and paranasal sinus mucosa $^{(85)}$. A study by Mott et al demonstrated a significant improvement in subjective and objective olfactory scores following topical nasal steroid spray in the head down position after 8-26 weeks treatment $^{(86)}$. Studies evaluating the effects of antibiotics and long term macrolides in the treatment of CRS and potentially olfaction are equivocal $^{(87)}$. Systemic corticosteroids have been shown to significantly improve hyposmia in patients with underlying sinonasal disease. Interestingly in the same study systemic steroids did not improve anosmia secondary to a viral insult suggesting a permanent damage to the olfactory mucosa $^{(88)}$.

**Alternative Therapies**

Alternative therapies such as herbal remedies, vitamin A, trace elements such as magnesium and zinc have been advocated by some studies and noted no improvement in olfactory function when zinc supplements were given to subjects $^{(81,89)}$. 

35
Head Injury

Patients with head injuries are the second largest group referred and account for 20% of anosmic patients seen in the out-patients. At present there does not exist a specific treatment for this group of patients. Studies have shown that a third of patients recover spontaneously which is thought to be due to regeneration of the olfactory system\(^\text{90}\). It has been shown that complete recovery can take up to five years\(^\text{90}\). Rombaux \textit{et al} showed that post-traumatic recovery of olfaction is highest in the first 6 months but continues for up to 2 years in addition demonstrating that an olfactory bulb volume of greater than 40mm correlated positively with recovery of sense of smell\(^\text{91}\).

Post viral

The third largest groups of patients are those with anosmia following an upper respiratory tract infection. They account for about for 15% of referrals. Equally there is not an effective medical treatment to restore the olfaction function in this group of patients\(^\text{92}\). Recovery occurs spontaneously in 50% of patients and occurs commonly six months after onset and the duration of recovery can be as long as 3 years\(^\text{79}\). Patients with anosmia for more than a year have been shown to have a poor prognosis in terms of recovery\(^\text{93}\).

Role of FESS in Olfactory dysfunction

Current evidence supports the efficacy of endoscopic sinus surgery in the significant improvement of olfactory dysfunction in CRS patients both in the short and long term and up to 5 years post operatively\(^\text{94}\). Additional studies have also shown improved olfactory outcomes following endoscopic sinus surgery albeit not always significant\(^\text{77,95}\). CRS sub group analysis of olfactory outcomes using psychophysical measurements in patients with CRSwNP and CRSSNP have been limited in numbers with regards to separate analysis for quality of life outcomes, olfactory function and other PROMs, however, a recent study evaluating CRS sub group analysis following endoscopic sinus surgery demonstrated a significant olfactory improvement in the CRSwNP sub group as measured by Sniffin sticks. This significant improvement was not demonstrated in the CRSSNP subgroup, although as a whole the general CRS population improved significantly\(^\text{96}\). Interestingly this olfactory outcome has not been evaluated using the UPSIT psychophysical olfactory measuring technique.
One of the earliest studies to assess olfaction following nasal surgery was performed by Kimmelman in 1994 (97). This study included 93 patients undergoing all types of nasal surgery: septal and turbinate surgery, rhinoplasty surgery, intranasal polypectomy and sinus surgery. The ‘University of Pennsylvania Smell Identification Test’ (UPSIT) was used and found 66% of patients either improved their sense of smell or remained unchanged following surgery. The remaining 34% showed a decline in olfaction with one patient becoming anosmic. This study forms the basis of the commonly quoted 1% risk of anosmia with nasal surgery. The cause for this anosmia remains unknown and may be due to anatomical alteration following surgery or chemosensory damage following the introduction of nasal medication. Lund and Scadding demonstrated a significant improvement in olfaction following FESS in a cohort of 200 patients (98).

Rowe-Jones et al, performed a 5 year prospective study on 109 post FESS CRS patients of which 75% were CRS with polyps and a significant improvement of olfaction was shown at 2 years post surgery which became non significant at 5 years (99). Jiang et al, measured olfaction using the UPSIT test, single threshold test and the discrimination test on 75 FESS patients and found irrespective of the type of olfactory test performed there was not a significant improvement (100).

Olfactory prognostic factors in efficacy of ESS

In a cohort of 330 CRS patients with olfactory dysfunction increasing age, nasal polyposis, smoking status and asthma were significant predictor factors for olfactory dysfunction whereas previous ESS or allergic rhinitis were not and equally septal deviation or inferior turbinate hypertrophy (101). Rudmik and Smith performed a literature review on the efficacy of FESS in CRS related olfactory dysfunction. It was concluded the evidence supporting its efficacy is equivocal and not significant. It was very difficult to predict olfactory outcomes following surgery and although a positive affect was more likely to occur, patients still remained hyposmic and there were some studies showing no change. It was extrapolated that patients who were hyposmic and had CRS with polyps stood the best chance of improvement and hence were good predictive factors (102).

A recent study by Shriever et al in 2013 looked at the effects of nasal surgery including both
sinus surgery and septal surgery on olfaction using the 16 item odor identification sniffing stick test. At 3.5 months post operatively there was a significant improvement in the sinus surgery arm as opposed to a non significant improvement in the septal surgery arm and interestingly they both became non significant at 12 months. They also found that polyps and eosinophilia were good prognostic factors for a significantly improved olfactory outcome. Conversely, in the non polyp sinus group the improvement was not significant (103). The effects of FESS and septal surgery on olfactory function are summarised in table 1.

Table 1 Effect of FESS and Septal Surgery on Olfactory function

<table>
<thead>
<tr>
<th>Author</th>
<th>Number of patients</th>
<th>Outcome</th>
</tr>
</thead>
</table>
| Kimmelman, 1994   | 93 patients including septal and sinus surgery | 66% Improved  
10% Declined  
1% Anosmia |
| Lund and Scadding, 1994 | 200 FESS patients | Significant improvement in olfaction following surgery |
| Rowe-Jones et al, 2005 | 109 FESS patients (75% polyps) | Significant olfactory improvement at 2 years and became non-significant at 5 years |
| Jiang et al, 2008  | 75 FESS patients | Non significant improvement in olfaction |
| Lind H et al, 2016 | 97 FESS patients | Significant improvement in the CRSwNP subgroup, and although improvement in the CRSsNP group was demonstrated it was non significant |
| Schriever et al 2013 | 157 FESS and septal surgery patients | Significant improvement in olfactory improvement at 3.5 months and non significant, but still improved at 12 |
CRS or not. CRSwNP a good prognostic factor, unlike CRSsNP

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients Described</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pade et al 2008</td>
<td>150 septal surgery patients</td>
<td>13% improvement only&lt;br&gt;81% no change&lt;br&gt;6% decrease</td>
</tr>
<tr>
<td>Randhawa et al 2016</td>
<td>43 functional Septorhinoplasty patients</td>
<td>A significant improvement in olfaction was demonstrated</td>
</tr>
<tr>
<td>Damm et al 2003</td>
<td>30 patients undergoing septoplasty and inferior turbinate surgery</td>
<td>80% had improved olfactory outcomes</td>
</tr>
<tr>
<td>Pfarr et al 2004</td>
<td>30 patients undergoing septoplasty surgery</td>
<td>No significant improvement in olfaction was seen</td>
</tr>
</tbody>
</table>

**NOSE score in the FESS population**

Up to now, the NOSE score which is a patient reported outcome measure (PROM) commonly used in septoplasty and septorhinoplasty surgery, has not been evaluated in the CRS population even though it has been recommended in CRS patients (65;104). CRS studies have demonstrated that nasal blockage VAS scores following ESS improve significantly and correlate with improved olfactory function on sniffing stick evaluation (105). The question remains whether a specific PROM looking at all aspects of nasal blockage (NOSE) equally improves following ESS and also correlates with improved olfactory function. Our aims were to determine the efficacy of endoscopic sinus surgery (ESS) on olfactory function in CRSwNP and CRSsNP sub groups and to further evaluate the nasal obstruction and symptom evaluation (NOSE) scale in the CRS population.

**Septorhinoplasty surgery and sense of smell**

The role of septorhinoplasty surgery in the treatment of olfactory disorders remains equally equivocal. In the majority of patients the sensorineural component of the olfactory pathway is
intact unlike in the CRS group and the conductive pathway is obstructed either through a deviated septum or reduced internal and external valve function \(^{(106)}\). Transient post-operative hyposmia is a common feature following external SRP and can require 6 weeks to 6 months for recovery to pre-operative olfactory function \(^{(107)}\). The impact of surgery upon olfactory function can also be effectively assessed using pre- and post-operative sinonasal outcome test (SNOT-22) scores. In this study olfaction as measured subjectively by the SNOT-22 improved significantly following Septorhinoplasty surgery \(^{(108)}\).

In a prospective study of 150 patients undergoing septal surgery, Pade et al assessed olfaction using ‘Sniffin’ Sticks’, and demonstrated a 13% improvement, no change in 81%, and a decreased olfactory function in 7% \(^{(109)}\). Shriever et al demonstrated in the septal arm after a long term follow up of 44 patients that a significant change in olfaction was not seen \(^{(103)}\). Damm et al, demonstrated that septoplasty and inferior turbinectomy caused a profound increase in supra-threshold odour identification, but not an increase in odour thresholds \(^{(110)}\). Interestingly, Pfaar et al, showed a contradictory result in their cohort of 25 patients whereby the supra-threshold odour identification did not improve but the odour threshold did \(^{(111)}\). In summary, with the available data available, there is a likelihood of improvement in the sense of smell following septorhinoplasty surgery.

Lateralised olfactory function or unilateral anosmia exists in 15% of healthy people \(^{(112)}\). A deviated septum has also been shown to reduce air entry and cause nasal obstruction but has also been shown to reduce olfactory function with a subsequent reduction in the size of the olfactory bulb \(^{(113)}\). Fyrampas et al demonstrated that patients with a nasal septal deviation showed higher olfactory thresholds on the convex side and the post operative scores following septoplasty surgery did not show a convincing increase in olfaction \(^{(114)}\). Randhawa et al demonstrated a statistically significant improvement in Sniffin stick olfactory identification following functional septorhinoplasty surgery \(^{(126)}\).

It is difficult to differentiate whether hyposmia is due to a conductive or sensorineural cause although in septorhinoplasty surgery the olfactory sensory component is more than likely to be functioning particularly on comparison with CRS patients. A case report describes the complete restoration of olfaction following a functional septorhinoplasty procedure prior to which the patient was anosmic. Hypothetically the olfactory regeneration may have been reinvigorated through repositioning the perpendicular plate as shown in figure 7 which is in
continuity with the crista galla and hence potentially stimulating the regeneration of the olfactory neurons (115).

**Figure 7**
The perpendicular plate and crista galli are in continuum and represent the area of transit for the olfactory bundles into the anterior skull base
Hypotheses and aims

The primary hypothesis states that olfactory harvesting is a patient safe procedure and does not incur an increase in complication rate and does not reduce nasal function including the sense of smell when compared to a control group. The secondary hypothesis states that ESS improves the olfactory outcome in CRS patients both with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP).

The additional aims of this thesis are to perform a subgroup analysis within the CRS cohort and determine whether there is a statistical significant difference in olfactory outcomes between CRS patients with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP) and also critique the effectiveness of the nasal obstruction symptom evaluation (NOSE) patient reported outcome measure (PROM) in the CRS population which has not been previously assessed. Overall, the aim is to better understand the effects of nasal olfactory biopsy surgery on olfaction as well as patient morbidity and secondly understand the effects of sinus surgery on olfactory function in the CRS subgroups whilst additionally evaluating the NOSE PROM.

The primary endpoint measure will be olfaction. This will be measured using the University of Pennsylvania Smell Identification Test (UPSIT). The secondary nasal function endpoints will be surgeon reported endoscopic outcomes (Lund-Kennedy Staging System), and patient reported quality of life (QOL) and symptom outcome measures including; Sino-Nasal Outcome test 22 (SNOT 22), Visual Analogue Scale (VAS) of nasal symptoms and the Nasal Obstruction and Symptom Evaluation scale (NOSE). This research is being undertaken in connection with our work on OEC culture techniques, with a view to perform clinical trials of nerve repair in the future.\textsuperscript{(4,23)}
CHAPTER 2

MATERIALS AND METHODS

Ethical Approval
Full Ethical and Research and Development (R&D) approval was sought by PA as the principal investigator for the RNTNEH arm of this trial following original approval by DC at the National Hospital for Neurology and Neurosurgery and Institute of Neurology joint research ethics committee. REC reference: 05/Q0512/103

Informed Consent
Patients were consented for participating in this study separately to their consent for the operation. The research consent form and patient information sheet were explained to the patient both by the Principal Investigator and research nurse. The aims of the study, risk factors of the procedure and the absence of direct benefit to the patients themselves were discussed prior to obtaining their consent. The risks of CSF leak and orbital damage were also outlined during consent and remedy surgery fully described. The patients were allowed 24 hours to reflect and reconsider. The consent form and patient information sheet were formulated and developed through utilizing Patient Public Involvement strategies as well as ‘patient forum groups’ based both at the National Hospital for Neurosurgery and Neurology and Royal National Throat Nose and Ear Hospital, Copies of the aforementioned forms are enclosed in the appendix.

Patient Recruitment

The 131 patients enrolled in this study were recruited from PA’s Rhinology clinic at the Royal National Throat Nose and Ear Hospital over a 2 year period. Those requiring Functional Endoscopic Sinus Surgery (FESS) for the treatment of a broad range of sinuse diseases as well as neoplasia were invited to participate in this prospective study. The exclusion criteria included patients aged under the age of 16, pregnancy and the inability to comprehend the assessment questionnaires.
**Table 2 Population data**

<table>
<thead>
<tr>
<th>Patients Data</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic Data</strong></td>
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</tr>
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</tr>
<tr>
<td>Sex, No (%)</td>
<td></td>
</tr>
<tr>
<td>♀ (41.2%)</td>
<td>54</td>
</tr>
<tr>
<td>♂ (58.8%)</td>
<td>77</td>
</tr>
<tr>
<td><strong>Surgical indication</strong></td>
<td>No (%)</td>
</tr>
<tr>
<td>CRSwNP with Polyps</td>
<td>55 (42.0%)</td>
</tr>
<tr>
<td>CRSsNP, without Polyps</td>
<td>42 (32.1%)</td>
</tr>
<tr>
<td>Antro-choanal Polyp</td>
<td>9 (6.9%)</td>
</tr>
<tr>
<td>Inverted papilloma</td>
<td>7 (5.3%)</td>
</tr>
<tr>
<td>Foreign body</td>
<td>4 (3.1%)</td>
</tr>
<tr>
<td>Fungocele / Mucocele</td>
<td>3 (2.3%)</td>
</tr>
<tr>
<td>Spheno-palatine ligation</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Ethmoidal adenocarcinoma</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Maxillary hypoplasia</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Oro-antral fistula</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Infected Concha bullosa</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>CSF leak</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Sphenoid fungus ball</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Pott's puffy tumour</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Frontal sinus stenosis</td>
<td>1 (1%)</td>
</tr>
<tr>
<td><strong>Comorbidities</strong></td>
<td>No (%)</td>
</tr>
<tr>
<td>Revision surgery</td>
<td>16 (12.2%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>26 (19.8%)</td>
</tr>
<tr>
<td><strong>CT-scan</strong></td>
<td>Lund-Mackay score, mean (± sd)</td>
</tr>
</tbody>
</table>
The patients recruited for this thesis reflect the tertiary referral nature of PA’s clinic with approximately 75% of the patients suffering with CRS as outlined in Table 2. The CRS patients described a combination of symptoms including nasal obstruction, reduction in their sense of smell, rhinorrhoea and pressure like facial pain. The cause for the CRS ranged from inflammatory nasal polyposis, viral, bacterial or fungal rhinosinusitis. We also recruited non-CRS patients with structural disorders as well as patients with benign and malignant tumours. The biopsies from the patients with neoplasia were harvested from the normal side.

The EPOS guidelines were adhered to prior to listing of patients for sinus surgery and the majority of our patients suffered with refractory chronic rhinosinusitis\(^{74}\). EPOS guidelines define rhinosinusitis (including nasal polyps) as inflammation of the nose and the paranasal sinuses characterized by two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip): +/- facial pain/pressure, +/- reduction or loss of smell; and either endoscopic signs of polyps and/or; mucopurulent discharge primarily from the middle meatus and/or CT changes of mucosal changes within the ostiomeatal complex and/or sinuses. The aim of Endoscopic Sinus Surgery (ESS) is to improve the ventilation of the sinuses with restoration of mucociliary clearance and enhancement of medical treatment.

**Patients Preoperative and Post Operative symptom Assessments**

Patients were evaluated pre-operatively, intra-operatively and post-operatively both in the immediate post operative recovery and subsequent post operative follow-up at 6 months. We compared our findings with a control group who equally underwent FESS surgery but without an olfactory biopsy. The patient’s medical history was documented including age, sex, presenting complaint, past medical and surgical history, drug history, allergies and smoking status. In addition, recent use of topical and/or oral steroids was documented.

The subjective patient reported outcome measurements (PROMs) used in this study included the SNOT 22 quality of life score, the NOSE score and VAS scores. Disease severity was also objectively assessed on CT scanning which was a surgical prerequisite. Disease severity
was graded according to opacification on CT scanning using the Lund and Mackay grading system. Disease severity was also assessed using the Lund-Kennedy surgeon reported outcome measure which grades nasal mucosa quality and the sense of smell was measured pre and post operatively using the UPSIT.

Post Operative morbidity

It was important to both patient and surgeon reported outcomes measures and although other olfactory measurements and nasal function PROMs could have been used in this study, we decided to focus on those we are more familiar with as well as their subsequent interpretation. The individual PROMs and surgeon reported outcome measures used in this study will be critiqued within the discussion.

Patient Safety was assessed through the evaluation of both intra operative and post operative complication rates which included bleeding rates, infection and CSF leakage. The immediate adverse safety outcomes evaluated during the biopsy were haemorrhage and cerebrospinal fluid leakage. This was assessed at the time of surgery and immediately post-operatively. Secondary haemorrhage and infection was assessed at the 3 week follow up appointment.

Radiology

The Lund and Mackay staging system scores severity of sinus opacification found on CT scanning. The staging system bilaterally scores the five sinuses from zero to two, depending on severity of opacification. A zero is assigned to a sinus without opacification and a two is assigned to complete opacification, and a one for partial opacification. The ostiomeatal complex (OMC) is also scored but either one or two (presence or absence of opacification). The grading score ranges from 0 to 24. Both CT and MRI images of the paranasal sinuses are depicted in figures 8, 9 and 10.
**Figure 8**

Coronal CT scan of paranasal sinuses showing partial opacification of the anterior ethmoidal sinuses and highlighting the inferior, middle and superior turbinates.
Figure 9
A normal coronal CT scan of the sinuses highlighting the right superior turbinate

Figure 10
T1 MRI scan highlighting the superior turbinates and their attachments to the anterior surface of the sphenoid sinuses
Lund and Kennedy Surgeon reported sinus outcome

The Lund-Kennedy endoscopic scoring system is a clinician reported outcome measure which is subjectively scored by the surgeon and quantifies the pathological state of the paranasal sinuses and in doing so quantifies the severity of polyps, discharge, edema, scarring, or adhesions and crusting and the score ranges from 0 to 20. Polyps are graded as absent (0), present in the middle meatus (1), or present beyond the middle meatus (2). Discharge is graded as not present (0), thin (1), or thick and purulent (2). Edema, scarring, and crusting are each graded as absent (0), mild (1), or severe (2)\(^{(117-119)}\). The appearance of normal sinonasal mucosa is depicted in figure 11.

Figure 11
Endoscopic photograph of the right nasal cavity, showing the right middle turbinate, middle meatus and exhibiting a normal appearance.
**Visual Analogue Scale**

This is a validated subjective scoring system used for discreet individual symptom scoring. The patient marks on a line from zero to ten their perceived severity and a scale of five has been shown to affect the quality of life of the patient. Visual analogue scales for smell and overall nasal symptoms were completed on a scale of 0-10 in terms of severity.

**Sino-Nasal Outcome Test (SNOT22)**

The SNOT-22 is a validated 22 question quality of life disease specific questionnaire which rates the severity of their symptoms from 0 (no problem) to 5 (problem as bad as it can be) and gives a theoretical total score of 110 (Figure 12) \(^{(120)}\).
Figure 12 SNOT 22 Questionnaire

<table>
<thead>
<tr>
<th></th>
<th>No problem</th>
<th>Very mild problem</th>
<th>Mild or slight problem</th>
<th>Moderate problem</th>
<th>Severe problem</th>
<th>Problem as bad as it can be</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Need to blow nose</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>2.</td>
<td>Sneezing</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>3.</td>
<td>Runny nose</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4.</td>
<td>Cough</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5.</td>
<td>Post nasal discharge excessive</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>6.</td>
<td>Thick nasal discharge</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>7.</td>
<td>Ear fullness</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>8.</td>
<td>Dizziness</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>9.</td>
<td>Ear pain/pressure</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>10.</td>
<td>Facial pain/pressure</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>11.</td>
<td>Difficulty falling asleep</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>12.</td>
<td>Waking up at night</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>13.</td>
<td>Lack of a good night's sleep</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>14.</td>
<td>Waking up tired</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>15.</td>
<td>Fatigue during the day</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>16.</td>
<td>Reduced productivity</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>17.</td>
<td>Reduced concentration</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>18.</td>
<td>Frustrated/restless/impatient</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>19.</td>
<td>Sad</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>20.</td>
<td>Embarrassed</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>21.</td>
<td>Sense of taste/smell</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>22.</td>
<td>Blockage/congestion of nose</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

TOTAL: ___ ___ ___ ___ ___
Nose Questionnaire

The nasal obstruction symptom evaluation (NOSE) questionnaire, as shown in figure 13, is a validated disease-specific instrument which assesses different components of nasal obstruction (104). It is commonly used in otolaryngology practices to provide an objective measure of nasal obstruction. The instrument is brief and easy to complete, with minimal respondent burden. It consists of 5 questions seeking to rate the burden of nasal obstruction during the past month scored from 0 (not a problem) to 4 (severe problem) giving a score of between 0-20. It is well validated and has been used to measure quality of life improvements in nasal septal surgery, functional septorhinoplasty and nasal valve surgery with good effect. Classically the final result is multiplied by five to give a maximum score of 100.

Figure 13

Nose questionnaire

<table>
<thead>
<tr>
<th></th>
<th>Not a Problem</th>
<th>Very Mild Problem</th>
<th>Moderate Problem</th>
<th>Fairly Bad Problem</th>
<th>Severe Problem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal stuffiness</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Nasal blockage or obstruction</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Trouble breathing through my nose</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Trouble sleeping</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Unable to get enough air through my nose during exercise or exertion</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>
**UPSIT**

The UPSIT is our screening test of choice and works on the scratch and sniff principle revolving around 40 different odors, as shown in figure 14. It tests cognition at the same time. The UPSIT test has been proven both reliable and reproducible as a simple clinical test of olfaction and is the most commonly used test. The raw UPSIT scores were calculated as the number of correct identifications, ranging from 0 to 40, with 40 representing perfect olfaction (121).

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**Figure 14**

UPSIT Questionnaire

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**Surgical Procedure**

The nose was prepared in the majority of cases with Moffatts solution (2mls of 6% cocaine, 1ml of 1:1000 epinephrine, 2mls of 8% sodium bicarbonate and made up to 10mls with 5mls of normal saline) and applied by the anaesthetist in the anaesthetic room prior to surgery.
Only very occasionally if the patient had cardiac morbidity was an alternative preparation used in the form of co-phenylecaine nasal spray (lignocaine hydrochloride 50mg/ml and phenylephrine hydrochloride 5mg/ml). The patients were positioned supine with a 20 degree head elevation using hypotensive general anaesthetic techniques. The nose was further decongested under endoscopic guidance by the surgeon through applying ribbon gauze soaked epinephrine (1 in 1000 epinephrine) into and around the middle meatus. The nasal cavity was simultaneously endoscopically assessed using a 4mm endoscope and undertaking the three pass technique and evaluated using the Lund Kennedy scoring system. The nasal mucosa was assessed and scored for oedema, discharge and polyposis as well as crusting and adhesions.

Topical adrenaline was further applied to the superior turbinate prior to the olfactory mucosa harvesting technique, following which the remaining endoscopic sinus surgery was completed. Post operative nasal packing was not used although a half inch ribbon gauze soaked in 1 in 1000 adrenaline was installed and removed in recovery before being transferred back to the ward. Patients were routinely discharged on the same day in the majority of cases. The harvesting procedure was performed using the standard FESS set which included endoscopic forceps and scissors as shown in figure 15.

Figure 15 Selection of endoscopic instruments
The post operative nasal medications included regular nasal douches with salt and sodium bicarbonate and followed up in out-patients at 3 weeks to check histology and to endoscopically decrust as required. Patients were then seen at 6 months post operatively and further evaluated both endoscopically using decongestant in the form of co-phenylcaine nasal spray with repeat PROMS and smell evaluation.

**Statistics and sample size**

Statistical tests were undertaken using Stata version 13.1 (StatCorp, Tx). Graphical presentations were performed using GraphPad Prism 5.0a (GraphPad Software, San Diego, California, USA).

The olfactory scores between groups were compared using 2-ways analysis of variance, with turbinate biopsy and endoscopic sinus surgery as a source of variation. Analysis was performed first on the raw UPSIT scores as the primary endpoint. Scores were also adjusted for age and gender using percentile norms published by Doty et al (121).

The impact of secondary endpoints (Lund-Kennedy endoscopic outcome, SNOT-22, VAS, NOSE score) were also sought by 2-ways ANOVA. Correlation between objective olfactory score assessed by UPSIT and subjective patient’s perception assessed by visual analogue scale on sense of smell was analysed using Spearman regression. Effects of cofactors such as smoking, sex and polyposis on baseline UPSIT scores were analysed using U Mann Whitney test. Linear regression analysis was used to calculate the correlation between age and the baseline UPSIT score. Interaction of cofactors on the olfactory results of sinus surgery was evaluated using 2-ways ANOVA.

**Sample Size Calculation**

All patients were consented for the procedure and refusal for the biopsy allowed for potential entry into the control arm. Sample size calculation of 41 patients in each arm was deemed satisfactory so as to provide a significant result although a larger sample was deemed ideal to
account for drop out. Using data from the pilot series of patients, 7 pre-op and 4 post-op UPSIT scores, average change in mean UPSIT is 0.33, sd 3.2, to detect a change difference in score change of 0.33 in control group and 3 in olfactory biopsy group, power 80%, P=0.05, requires 31 patients in pre-op and post-op groups. Allowing for 10 patients loss to follow-up, a sample size of 41 patients was required for each group.

Estimated sample size for two-sample comparison of means; Test Ho: m1 = m2, where m1 is the mean in population 1 and m2 is the mean in population 2, assumptions: alpha = 0.0500 (two-sided) power = 0.9000, m1 = .33, m2 = 3, sd1 = 3.2 and sd2 = 3.2, n2/n1 = 1.00 and estimated required sample sizes: n1 = 31 and n2 = 31.

**Funding**

Funding was received from the European Research Council (Ref: HORAB, LS9, ERC-2009-StG). This work was also conducted, in part, in the UCL Institute of Neurology, which receives funding from the University College London Hospital, National Institute of Health Research, Comprehensive Biomedical Research Centre.
CHAPTER 3

RESULTS

Surgical modifications of the superior turbinate harvesting technique

The preliminary findings of this harvesting technique have been published although the resultant modifications have not been documented \(^{(4)}\). This technique has been modified so as to ensure minimal patient morbidity particularly with regards to anterior skull base injury and CSF leak as well as significant bleeding. During this modification process only one reported CSF leak was reported and was immediately apparent. The defect was subsequently repaired with a fat plug and a fascia lata with free mucosal graft reconstructive techniques. The patient made a full and beneficial recovery. In this particular case the superior portion of the superior turbinate was being trialed and it was immediately apparent the anterior skull base had been breached with an immediate CSF leak. As a result the superior section was abandoned and all subsequent biopsies included the middle portion of the superior turbinate. The superior section of the superior turbinate lies in close proximity to the skull base.

The key with this biopsy technique is to include the whole of the middle section ensuring the turbinate bone remains in-situ so as to act as a scaffold and allow for orientation of the nasal mucosa. It became apparent during the modification phase of our harvesting technique that histological preparation of the biopsy was made easier when the mucosa was still attached and orientated onto the underlying turbinate bone and lamina propria. In figure 16 the left superior turbinate can be seen with the sphenoid ostium visualised medially and the procedure performed as outlined in figures 17 to 20 with image guidance as outlined in figures 21 to 31.
**Figure 16**
Left superior turbinate can be seen with the patent sphenoid ostium visualised medially adjacent to the septum on the left.

**Figure 17**
Endoscopic micro scissors are shown making the superior horizontal incision.
**Figure 18**
Inferior horizontal incision to the superior turbinate is fashioned.

**Figure 19**
The inferior incision is joined vertically to the superior horizontal incision and the middle section is removed gently with a blakesley forceps.
Image Guidance Delineation of skull Base

The anterior skull base was delineated during this process using intra operative image guidance which enabled accurate visualization of the superior turbinate and its skull base attachment. The superior nasal cavity starts anteriorly underneath the nasal and frontal bones as shown in figure 21 and progresses onto the skull base more posteriorly as highlighted in figure 22. The root of the right middle turbinate can just been seen in the bottom right of the figure. In figure 23 the root of the right middle turbinate is visualized and its image guidance image is superimposed on figure 24 showing its close proximity to the anterior skull base.
Figure 21
Roof of nasal cavity underneath the nasal bones

Figure 22
Olfactory niche and the demarcation between under surface of nasal bones and skull base. The septum is medial and the middle turbinate lateral.
Figure 23
Imaging guidance probe on root of middle turbinate

Figure 24
Superimposed image guidance view of skull base and the root of the middle turbinate
Figure 25
Endoscopic view of the right superior turbinate

In figure 25, the superior turbinate is seen in the background. In figure 26 the middle section of the superior turbinate is probed and super imposed with image guidance navigation which highlights its relationship with the skull base and sphenoid sinus.
Figure 26
Superimposed Image guidance navigation of the superior turbinate as shown in figure 24 and its relationship with the skull base.
Figure 27
Image guidance probe sited on the entrance of the right frontal sinus
Figure 28
Super-imposed Image guidance navigation image from figure 27 of the right frontal sinus.
Figure 29
Endoscopic image of the left superior turbinate as seen from the right nasal passage following the removal of posterior septum (septectomy)
Figure 30
Endoscopic image of the right ethmoidal nerve just anterior to the olfactory prominence and posterior to the frontal sinus ostium
Image guidance

The first olfactory nerve innervates the olfactory mucosa of the superior septum. It also demarcates the anterior boundary of the anterior skull base and therefore an important surgical landmark which is commonly used during the endoscopic modified Lothrop procedure or Draf 3 technique \(^{(57)}\). The anterior ethmoidal sensory nerve is often seen before the first olfactory nerve and lies just anterior to the skull base as shown in figure 30.
Table 3 **Comparison of the Population with and without Sup’ turbinate biopsy**

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<tr>
<th>Patients Data</th>
<th>Biopsy</th>
<th>No biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic data</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total, No</td>
<td>65</td>
<td>66</td>
</tr>
<tr>
<td>Age, mean (± sd), yr</td>
<td>47.2 (±14.5)</td>
<td>46.7 (±15.9)</td>
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<td>Sex, No (%)</td>
<td>24 ♀ (36.9%)</td>
<td>30 ♀ (45.4%)</td>
</tr>
<tr>
<td></td>
<td>41 ♂ (63.1%)</td>
<td>36 ♂ (54.6%)</td>
</tr>
<tr>
<td><strong>Surgical indication</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRS with Polyps, No (%)</td>
<td>23 (35.4%)</td>
<td>32 (48.5%)</td>
</tr>
<tr>
<td>CRSsP, No (%)</td>
<td>21 (32.3%)</td>
<td>21 (31.8%)</td>
</tr>
<tr>
<td>Antro-choanal Polyp, No (%)</td>
<td>6 (9.2%)</td>
<td>3 (4.5%)</td>
</tr>
<tr>
<td>Inverted papilloma</td>
<td>5 (7.7%)</td>
<td>2 (3.0%)</td>
</tr>
<tr>
<td>Foreign body, No (%)</td>
<td>3 (4.6%)</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td>Fungocele / Mucocele, No (%)</td>
<td>2 (3.1%)</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td>Spheno-palatine ligation, No (%)</td>
<td>0 (0.0%)</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td>Amyloidosis, No (%)</td>
<td>1 (1.5%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Sarcoidosis, No (%)</td>
<td>0 (0.0%)</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td>Ethmoidal adenocarcinoma, No (%)</td>
<td>1 (1.5%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Maxillary hypoplasia, No (%)</td>
<td>0 (0.0%)</td>
<td>1 (1.5%)</td>
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<tr>
<td>Oro-antral fistula, No (%)</td>
<td>0 (0.0%)</td>
<td>1 (1.5%)</td>
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<tr>
<td>Infected Concha bullosa, No (%)</td>
<td>1 (1.5%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>CSF leak, No (%)</td>
<td>0 (0.0%)</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td>Sphenoid fungus ball</td>
<td>0 (0.0%)</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td>Pott's puffy tumour</td>
<td>1 (1.5%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Frontal sinus stenosis</td>
<td>1 (1.5%)</td>
<td>0 (0.0%)</td>
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<tr>
<td><strong>Comorbidities</strong></td>
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<td>Revision surgery, No (%)</td>
<td>8 (12.3%)</td>
<td>8 (12.1%)</td>
</tr>
<tr>
<td>Smoking, No (%)</td>
<td>16 (24.6%)</td>
<td>10 (15.2%)</td>
</tr>
<tr>
<td><strong>CT-scan</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lund-Mackay score, mean (± sd)</td>
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<td>11.8 (±6.9)</td>
</tr>
<tr>
<td>Surgical Response</td>
<td>pre-operative</td>
<td>post-operative</td>
</tr>
<tr>
<td>-------------------</td>
<td>---------------</td>
<td>----------------</td>
</tr>
<tr>
<td><strong>UPSIT (value)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole population, mean (± sd)</td>
<td>25.9 (±9.6)</td>
<td>26.7 (±9.7)</td>
</tr>
<tr>
<td>Superior turbinate biopsy</td>
<td>26.6 (±9.3)</td>
<td>27.9 (±8.8)</td>
</tr>
<tr>
<td>No biopsy</td>
<td>25.2 (±9.9)</td>
<td>25.1 (±10.7)</td>
</tr>
<tr>
<td><strong>UPSIT (percentile)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole population, mean (± sd)</td>
<td>17.5 (±23.3)</td>
<td>19.4 (±22.3)</td>
</tr>
<tr>
<td>Superior turbinate biopsy</td>
<td>17.0 (±19.4)</td>
<td>20.1 (±21.6)</td>
</tr>
<tr>
<td>No biopsy</td>
<td>18.0 (±26.9)</td>
<td>18.8 (±22.7)</td>
</tr>
<tr>
<td><strong>SNOT-22 score</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole population, mean (± sd)</td>
<td>51.9 (±25.4)</td>
<td>29.1 (±24.2)</td>
</tr>
<tr>
<td>Superior turbinate biopsy</td>
<td>54.8 (24.5)</td>
<td>32.7 (±26.9)</td>
</tr>
<tr>
<td>No biopsy</td>
<td>48.9 (±26.2)</td>
<td>25.1 (±20.3)</td>
</tr>
<tr>
<td><strong>Lund-Kennedy score</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole population, mean (± sd)</td>
<td>4.4 (±2.7)</td>
<td>2.1 (±1.6)</td>
</tr>
<tr>
<td>Superior turbinate biopsy</td>
<td>4.2 (±2.7)</td>
<td>2.5 (±1.8)</td>
</tr>
<tr>
<td>No biopsy</td>
<td>4.6 (±2.7)</td>
<td>1.5 (±1.3)</td>
</tr>
<tr>
<td><strong>VAS score on overall nose symptom</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole population, mean (± sd)</td>
<td>6.5 (±2.8)</td>
<td>3.3 (±2.7)</td>
</tr>
<tr>
<td>Superior turbinate biopsy</td>
<td>6.8 (±2.6)</td>
<td>3.4 (±3.1)</td>
</tr>
<tr>
<td>No biopsy</td>
<td>6.2 (±3.0)</td>
<td>3.3 (±2.2)</td>
</tr>
<tr>
<td><strong>VAS score on sense of smell</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole population, mean (± sd)</td>
<td>5.9 (±3.5)</td>
<td>3.6 (±3.3)</td>
</tr>
<tr>
<td>Superior turbinate biopsy</td>
<td>5.6 (±3.4)</td>
<td>3.6 (±3.3)</td>
</tr>
<tr>
<td>No biopsy</td>
<td>6.3 (±3.7)</td>
<td>3.7 (±3.4)</td>
</tr>
<tr>
<td><strong>NOSE score</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall, mean (± sd)</td>
<td>12.9 (±5.9)</td>
<td>5.6 (±4.9)</td>
</tr>
<tr>
<td>Superior turbinate biopsy</td>
<td>13.8 (±5.9)</td>
<td>5.8 (±5.5)</td>
</tr>
<tr>
<td>No biopsy</td>
<td>12.3 (±5.9)</td>
<td>5.3 (±4.3)</td>
</tr>
</tbody>
</table>
Table 5 Effect of the sup’ turbinate biopsy on UPSIT value

Analysis by 2-way ANOVA.
Biopsy has no interaction with FESS (p=0.6376).
Biopsy does not affect the UPSIT value (p=0.1468).
FESS does not affect the UPSIT value (p=0.7032).
Table 6 **Effect of the sup’ turbinate biopsy on UPSIT percentile**

Analysis by 2-way ANOVA.
Biopsy has no interaction with FESS (p=0.7492).
Biopsy does not affect the UPSIT percentile (p=0.9693).
FESS does not affect the UPSIT percentile (p=0.5659).
Table 7 Effect of the sup’ turbinate biopsy on SNOT-22

Analysis by 2-way ANOVA.
Biopsy has no interaction with FESS (p=0.9368).
Biopsy does not affect the SNOT-22 (p=0.0921).
FESS affects the SNOT-22 significantly (p<0.0001) *** (improvement).
Table 8  **Effect of the sup’ turbinate biopsy on Lund-Kennedy endoscopic score**

Analysis by 2-way ANOVA.

Biopsy and FESS interact on the Lund-Kennedy score (p=0.0416).

Biopsy does not affect the Lund-Kennedy endoscopic score (p=0.3724).

FESS affects the Lund-Kennedy endoscopic score significantly (p<0.0001) ***
Table 9  **Effect of the sup’ turbinate biopsy on overall Visual Analogue Scale**

Analysis by 2-way ANOVA.
Biopsy has no interaction with FESS (p=0.5944).
Biopsy does not affect the overall VAS (p=0.4534).
FESS affects the overall VAS significantly (p<0.0001) *** (improvement).
Table 10 **Effect of the sup’ turbinate biopsy on the NOSE score**

Analysis by 2-way ANOVA.
Biopsy has no interaction with FESS (p=0.6091).
Biopsy does not affect the NOSE score (p=0.3291).
FESS affects the NOSE score significantly (p < 0.0001) *** (improvement).
Table 11 **Effect of the sup’ turbinate biopsy on Visual Analogue Scale on sense of Smell**

Analysis by 2-way ANOVA.
Biopsy has no interaction with FESS (p=0.6835).
Biopsy does not affect the VAS on sense of Smell (p=0.5920).
FESS affects the VAS on sense of Smell significantly (p<0.0001) *** (improvement).
Table 12 Is there a correlation between the Visual Analogue Score on sense of Smell and the UPSIT value?

No correlation (Spearman $R^2 = 0.2332$)
Table 13 **Is there a correlation between the Visual Analogue Score on sense of Smell and the UPSIT percentile?**

No correlation (Spearman $R^2 = 0.2398$)
Table 14 **Effect of Polyps on UPSIT value**

Analysis by Mann-Whitney test.
Patients with polyps have significantly lower UPSIT results pre-operatively (p<0.0001)***. 
Table 15 **Effect of polyps on UPSIT value, Analysis by 2-way ANOVA.**
Polyps have no interaction with FESS on UPSIT results (p=0.3848).
Polyps affect significantly the UPSIT value (p<0.0001) ***.
FESS does not affect the UPSIT value (p =0.4477).
Table 16 **Effect of Polyps on UPSIT percentile**

Analysis by Mann-Whitney test.
Patients with polyps have significantly lower UPSIT percentile pre-operatively (p<0.0001)***.
Table 17 **Analysis by 2-way ANOVA.**
Polyps have no interaction with FESS on UPSIT percentile (p=0.8227).
Polyps affect significantly the UPSIT percentile (p<0.0001) ***.
FESS does not affect the UPSIT percentile (p =0.7036).
Table 18 Effect of Smoking on UPSIT value

Analysis by Mann-Whitney test.
No difference between smokers and non-smokers on the UPSIT results pre-operatively (p=0.4213).
Table 19 **Analysis by 2-way ANOVA.**

Smoking has no interaction with FESS on UPSIT results (p=0.2834).
Smoking could affect UPSIT value (p=0.0320).
FESS does not affect UPSIT value (p =0.2938).

![Bar chart showing UPSIT value comparison between Smoking and Non Smoking before and after surgery.](Image)
Table 20  **Effect of Smoking on UPSIT percentile**

Analysis by Mann-Whitney test.
No difference between smokers and non-smokers on the UPSIT percentile pre-operatively (p=0.9615).

![Graph showing data for smokers and non-smokers on UPSIT percentile](image)

\[ p = 0.9615 \]
Table 21 **Analysis by 2-way ANOVA.**

Smoking has no interaction with FESS on UPSIT percentile (p=0.1395).

Smoking does not affect UPSIT percentile (p=0.3299).

FESS does not affect UPSIT percentile (p =0.1888).
Table 22 **Effect of the age on UPSIT value**

No correlation between age and UPSIT value
Table 23 **Effect of gender on UPSIT value**

Analysis by Mann-Whitney test.
No difference between male and female on the UPSIT results pre-operatively (p=0.2222).

![Scatter plot showing UPSIT values for male and female, with p = 0.2222.]

![Bar chart showing UPSIT values for male and female, with p = 0.2222.]

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Table 24 **Patient Data on subgroup analysis within the CRS cohort of patients**

<table>
<thead>
<tr>
<th>Patients Data</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic Data</strong></td>
</tr>
<tr>
<td>Total, No</td>
</tr>
<tr>
<td>Age, mean (± sd), yr</td>
</tr>
<tr>
<td>Sex, No (%)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Clinical Data</strong></td>
</tr>
<tr>
<td>Polyposis, No (%)</td>
</tr>
<tr>
<td>Revision surgery, No (%)</td>
</tr>
<tr>
<td>Smoking, No (%)</td>
</tr>
<tr>
<td><strong>CT-scan</strong></td>
</tr>
<tr>
<td>Lund-Mackay score, mean (± sd)</td>
</tr>
</tbody>
</table>
**Table 25 Outcome measures on subgroup analysis within the CRS cohort of patients**

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>pre-operative</th>
<th>post-operative</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UPSIT (value)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole population, mean (± sd)</td>
<td>25.5 (±9.9)</td>
<td>26.6 (±9.7)</td>
<td>0.38 ns</td>
</tr>
<tr>
<td>CRSsNP</td>
<td>29.5 (±7.8)</td>
<td>29.1 (±8.1)</td>
<td>0.34 ns</td>
</tr>
<tr>
<td>CRSwNP</td>
<td>21.9 (±10.4)</td>
<td>24.3 (±10.5)</td>
<td>0.04 *</td>
</tr>
<tr>
<td><strong>NOSE score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole population, mean (± sd)</td>
<td>61.2 (±30.6)</td>
<td>27.8 (±25.4)</td>
<td>&lt;0.01 ***</td>
</tr>
<tr>
<td>CRSsNP</td>
<td>54.2 (±32.7)</td>
<td>31.8 (±27.2)</td>
<td>0.01 *</td>
</tr>
<tr>
<td>CRSwNP</td>
<td>67.2 (±27.7)</td>
<td>22.7 (±4.7)</td>
<td>&lt;0.01 ***</td>
</tr>
<tr>
<td><strong>SNOT-22 score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole population, mean (± sd)</td>
<td>53.4 (±24.8)</td>
<td>28.9 (±23.8)</td>
<td>&lt;0.01 ***</td>
</tr>
<tr>
<td>CRSsNP</td>
<td>52.8 (±26.3)</td>
<td>32.5 (±25.4)</td>
<td>&lt;0.01 ***</td>
</tr>
<tr>
<td>CRSwNP</td>
<td>54.0 (±23.7)</td>
<td>25.8 (±22.2)</td>
<td>&lt;0.01 ***</td>
</tr>
<tr>
<td><strong>Lund-Kennedy score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole population, mean (± sd)</td>
<td>4.6 (±2.7)</td>
<td>2.1 (±1.7)</td>
<td>&lt;0.01 ***</td>
</tr>
<tr>
<td>CRSsNP</td>
<td>3.2 (±2.3)</td>
<td>2.1 (±1.4)</td>
<td>0.15 ns</td>
</tr>
<tr>
<td>CRSwNP</td>
<td>5.7 (±2.6)</td>
<td>2.1 (±1.8)</td>
<td>&lt;0.01 ***</td>
</tr>
<tr>
<td><strong>VAS score on overall nose symptom</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole population, mean (± sd)</td>
<td>6.6 (±2.8)</td>
<td>3.4 (±2.8)</td>
<td>&lt;0.01 ***</td>
</tr>
<tr>
<td>CRSsNP</td>
<td>6.3 (±2.7)</td>
<td>4.4 (±2.8)</td>
<td>0.04 *</td>
</tr>
<tr>
<td>CRSwNP</td>
<td>6.9 (±2.8)</td>
<td>2.4 (±2.5)</td>
<td>&lt;0.01 ***</td>
</tr>
<tr>
<td><strong>VAS score on sense of smell</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole population, mean (± sd)</td>
<td>6.0 (±3.5)</td>
<td>3.7 (±3.4)</td>
<td>&lt;0.01 ***</td>
</tr>
<tr>
<td>CRSsNP</td>
<td>4.4 (±3.5)</td>
<td>2.5 (±3.0)</td>
<td>0.05 ns</td>
</tr>
<tr>
<td>CRSwNP</td>
<td>7.4 (±2.8)</td>
<td>5.0 (±3.4)</td>
<td>&lt;0.01 ***</td>
</tr>
</tbody>
</table>
Table 26 Comparison of SNOT22 and UPSIT on subgroup analysis within the CRS cohort of patients

Table Interpretation

Our population cohort, summarized in Table 2, was representative of the population undergoing sinus surgery in a tertiary referral hospital. Out of the 136 patients for whom inclusion in our study was offered, 131 completed the UPSIT test and were randomized for superior turbinate biopsy, 2 could not complete the UPSIT test due to language barrier, 1 did not have time to complete the UPSIT test, 1 patient declined to participate and 1 planned intervention was cancelled. Out of the 131 subjects included in our study, 65 underwent a superior turbinate biopsy and 66 were randomized for the control group. Demographic data,
severity of disease, co morbidities and surgical indications were comparable in the two groups.

There was no difference demonstrated between the groups for baseline UPSIT scores. Biopsy of the superior turbinate did not affect the UPSIT result (p=0.1468) and had no interaction with sinus surgery (p=0.6376). Sinus surgery failed to improve the UPSIT results in all patients (p=7032), with no difference between biopsy and control groups. Adjustment for age and sex using UPSIT percentile did not affect these findings. However, the evaluation of the sense of smell on a visual analogue scale subjectively improved in both groups after sinus surgery.

There was no significant difference between groups in terms of secondary endpoint measures (Lund-Kennedy endoscopic outcome, SNOT-22, VAS, NOSE score). There were no differences between the groups with respect to quality of life, patient’s reported olfactory measure or endoscopic evaluation. However, there was no correlation between the subjective sense of smell reported on a visual analogue scale and the objective evaluation by UPSIT. Superior turbinate biopsy did not affect the subjective sense of smell (p=0.5920) and had no interaction with sinus surgery (p=0.6835). Sinus surgery was able to improve significantly the subjective sense of Smell (p<0.0001) in both groups, independently of superior turbinate biopsy. Patients with nasal polyps had significantly lower baseline olfactory scores compared to patients without nasal polyps, but no effect of biopsy was detected between the groups on any of the outcome measures.

On sub group analysis of the CRS cohort, the population was consistent with the patients undergoing sinus surgery in a tertiary referral hospital (Table 24). Out of the 128 patients for whom inclusion in our study was offered, 113 agreed to enter the study. Half of the patients had chronic rhinosinusitis with nasal polyps. Mean Lund-Mackay score was 10.9 (severe chronic sinusitis on CT scan). The Lund-Mackay score was higher in the CRSwNP patient group (mean 14.9 ± 5.8) compared to the CRSsNP group (mean 6.4 ± 5.3). Mean Lund-Mackay score severity was not correlated to the UPSIT baseline score. However, patients with nasal polyps had lower baseline UPSIT scores compared to patients without polyps (p<0.0001). Through analyzing correlations between UPSIT and scores derived from the Lund Mackay (LM) and Lund-Kennedy (LK) grading systems a negative correlation was found for all scores preoperatively (Spearman r=-0.55 for LM scores and r=-0.50 for LK
scores). The UPSIT score was not significantly different in smoking or non-smoking patients in our population. Equally, in our population, age was not correlated to the baseline UPSIT score. The influence of chronic rhinosinusitis on olfaction measured by UPSIT was likely to be stronger than the effect of age and tobacco in our population.

The outcomes as summarized in table 25 demonstrate that the UPSIT score in the CRSwNP subgroup improved significantly after endoscopic sinus surgery (Wilcoxon p = 0.0428). A similar significant improvement in the VAS ‘sense of smell’ measurement was demonstrated in the CRSwNP subgroup (Wilcoxon p = 0.0004). However, although the UPSIT and VAS ‘sense of smell’ outcomes improved in the CRSsNP subgroup they were not significant. A significant improvement in the subjective olfaction scored by the visual analogue scale was also seen in the general CRS population (Wilcoxon p <0.0001). However, in the whole CRS population, olfactory improvement as measured by UPSIT was not significant after surgery. The UPSIT score did not change following surgery in 12.7% of the CRS cohort, it improved in 46.0% and worsened in 41.3%. However, the outcomes differed on perceived evaluation of olfaction as rated on the VAS ‘sense of smell’ measurement whereby it improved in 72.5% of the cohort, did not change in 13.7% patients and worsened in 13.7% patients following surgery. The SNOT-22 score, the surgeon reported endoscopic evaluation, the NOSE score and the visual analogue scale improved significantly after surgery in the whole population. The quality of life measured by SNOT-22 improved in 84.5% patients and decreased in 15.5% patients after surgery.

When reviewing the subgroups, baseline scores were seen to be worse in the polyp subgroup, but surgical improvement was especially marked in this subgroup. Among polyp patients, 54.5% improved their UPSIT score, while 12.1% did not change and 33.3% worsened after surgery. Subjective olfaction was even better with 81.4% of polyp patients reporting an improvement on visual analogue scale, 14.8% patients without change and 3.7% patients with worsening of olfaction. Improvement of quality of life was observed in 90.9% of polyp patients. The SNOT-22 decreased in 9.1% of the polyp patients.

SNOT-22 change and UPSIT change after surgery were correlated. More than 30% of the SNOT-22 variance was related to UPSIT improvement after surgery in polyps patients (r² = 0.379, p<0.001, Table 26). A correlation between the ‘sense of smell’ Visual Analogue Score and the UPSIT value was not demonstrated (Spearman R² = 0.13) A correlation between the
NOSE scale and UPSIT score ($r^2 = 0.01, p = 0.402$) equally was not demonstrated. There was no relationship between NOSE change after surgery and UPSIT change after surgery ($r^2 = 0.223, p = 0.0032$). A correlation between SNOT-22 change (Y-axis) and UPSIT change after surgery (Y-axis) in the CRSwNP was demonstrated in Table 26. SNOT-22 change and UPSIT change after surgery were fairly correlated, and more than 30% of the SNOT-22 variance was related to UPSIT improvement after surgery.
Table 27 Morbidity rates following surgery; primary and secondary haemorrhage and CSF leakage.

<table>
<thead>
<tr>
<th>Date</th>
<th>FESS</th>
<th>FESS Bx</th>
<th>Primary Haemorrhage</th>
<th>Secondary Haemorrhage</th>
<th>CSF leak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept/Oct 2012</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1 (Bx)</td>
</tr>
<tr>
<td>Nov/Dec 2012</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Jan/Feb 2013</td>
<td>9</td>
<td>8</td>
<td>0</td>
<td>1 Bx</td>
<td>0</td>
</tr>
<tr>
<td>Mar/Apr 2013</td>
<td>5</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>May/June 2013</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>July/Aug 2013</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sept/Oct 2013</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>1 (non biopsy)</td>
<td>0</td>
</tr>
<tr>
<td>Nov/Dec 2013</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Jan/Feb 2014</td>
<td>7</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mar/Apr 2014</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

According to Table 27 the primary haemorrhage rate was zero percent in both groups and the incidence of secondary haemorrhage rates was less than 1% in each group. The incidence of CSF leak was less than 1% in the biopsy group if the single superior segment biopsy is also included in this analysis but is zero percent if only the middle section harvesting technique is included. A significant difference in complication rates between the two groups was not demonstrated on Mann-Whitney evaluation p<0.05%.
CHAPTER 4

DISCUSSION

The primary hypothesis which stated that olfactory harvesting is ‘patient safe’ and does not incur a detriment to the patient’s nasal function and specifically does not reduce the patient’s sense of smell has been accepted as demonstrated by the results of this thesis. In addition we have demonstrated that patient morbidity and beneficial outcomes following harvesting human olfactory nasal mucosa during ESS is statistically the same compared to the outcomes of standard ESS without biopsy in this prospective controlled level 2b evidence based study.

The secondary hypothesis which stated that the effect of sinus surgery improves olfaction in both the CRSwNP and CRSsNP subgroups has also been accepted as demonstrated by the results of this thesis. In the CRSwNP subgroup, the patient’s perceived and measured sense of smell improved significantly following ESS and represents the most surgically responsive CRS subgroup as well as the most likely subgroup to improve the patient’s quality of life following an improved post operative olfactory response. This statistically significant olfactory improvement following surgery was not demonstrated in the CRSsNP subgroup. In addition we have uniquely demonstrated that the ‘NOSE’ patient reported outcome measure is a novel and sensitive outcome measure in the CRS population, however, it does not correlate with improved olfaction.

Patient safety and nasal function, including olfactory outcomes, were not affected by unilateral resection of the middle section of the superior turbinate when compared to the outcomes of standard endoscopic sinus surgery without an olfactory biopsy. Importantly we have uniquely demonstrated that the patient’s quality of life and nasal patency remain unaffected following olfactory mucosa harvesting. This level 2b evidence analysis has not been performed before and will aid in the process of informed consent as well as promote future harvesting developments.

One of our main concerns following the resection of the middle section of the superior turbinate was a potential reduction in the patient’s sense of smell owing to the inevitable loss
of olfactory mucosa. A perceived or measured olfactory loss was not demonstrated in this study and hence we can assume the olfactory loss was not significant enough for the patient to notice, which is in keeping with previous smaller studies \(^{(5;123)}\). Interestingly the perceived olfactory ability from healthy individuals and rhinological patients is generally poor. A recent study demonstrated even amongst healthy well functioning cognitive middle aged adults that 79% of this sample, with objectively assessed olfactory dysfunction, reported normal olfactory function \(^{(124)}\), a similar but less dramatic finding was also found in patients with rhinological pathology whereby 33% of patients felt had a normal sense of smell actually were hyposmic \(^{(76)}\).

The need to harvest olfactory tissue without causing harm to the patient was fundamentally evaluated in this thesis. The ongoing dilemma, on one hand, is the need to optimize yield rates of olfactory tissue, in particular OEC yield rates and yet on the other hand it’s to ensure patient safety and not disadvantage the donor with reduced nasal function ability. We have already demonstrated in previous work that OEC yield rates are higher when biopsies are harvested closer to the skull base as well as in younger patients with less sino-nasal disease however subsequent patient safety has not been robustly evaluated \(^{(4)}\). In this thesis it was vital to have this real time information on OEC yield rates whilst at the same time optimising our harvesting technique.

Although the harvesting of the middle section of the superior turbinate is shown to be safe there were initial problems during its development. In the early stages of the harvesting technique the superior aspect of the superior turbinate was trialed and this encroached directly onto the skull base with an immediate CSF leakage. This was the only CSF leak encountered during the study and the superior aspect of the superior turbinate was immediately abandoned. The defect was reconstructed with fascia lata and a free mucosal flap taken from the inferior turbinate. The patient was discharged home without further complications and made a full and beneficial recovery. As shown in figure 32 the superior section of the superior turbinate is in close proximity with the skull base and olfactory bulb and therefore will inevitably result in a CSF leak if harvested. It has been acknowledged that the evolution of this technique, for good science engineering reasons, may have confounded the results to a minor degree, however the majority of the biopsies did involve the middle section of the superior turbinate.
In this study the patient’s perceived and measured sense of smell were statistically unaffected following their olfactory biopsy. Interestingly, the patient’s subjective sense of smell improved significantly in both surgical groups whereas their measured or psychophysical sense of smell equally also improved but not significantly. A correlation between the patient’s UPSIT outcomes and their respective smell VAS scores was not found. It would therefore appear that the size of our olfactory biopsy and subsequent loss of olfactory tissue did not impact significantly on the remaining olfactory function. The average surface area of our olfactory biopsy measured 2 by 2 mm in size (4mm squared) and included the whole of the middle section of the superior turbinate. As previously described the average surface area of the human olfactory mucosa surface area is approximately 10 to 20mm squared in each
cavity. According to Kachramanoglou the average yield rate of OECs was 7.5% with an overall OEC cellular proportion of 25% (4). Assuming on average 25% of the harvested area contained olfactory mucosa then this would equate to a potential histological loss of 1mm squared from a potential olfactory maximum of 40mm squared. This equates to less than 5% of the total olfactory mucosa being harvested.

The alternative theory would argue that the biopsied olfactory area had regenerated and repopulated the olfactory epithelium however this argument is doubtful owing to the lack of regeneration of the biopsied middle section of the superior turbinate as seen in figure 33. In this figure you can see the deficient middle section with a small adhesion as seen endoscopically 2 years post operatively. The mucosa has regenerated over the defect but the surface area has remained reduced owing to the lack of turbinate architecture. The question remains as to what is the maximum amount of olfactory tissue which could be harvested without incurring a functional loss.
On subgroup analysis within our CRS patient cohort, the UPSIT psychophysical measurement significantly improved following endoscopic sinus surgery (ESS) in the CRSwNP subgroup as well as the patient’s perceived VAS sense of smell. However, in the CRSsNP subgroup, the improved VAS and UPSIT measurements were not significant. Hence, the CRSwNP subgroup represents the most surgically responsive group to improved olfaction.

Whilst the total CRS population, including both CRSwNP and CRSsNP subgroups, olfactory improvement as measured by the UPSIT was not significant after surgery whereas the VAS sense of smell score did improve significantly. However, the UPSIT and VAS ‘sense of smell’ evaluations did not correlate. Interestingly, the SNOT 22 outcome significantly improved in both subgroups following surgery and importantly a positive relationship between improved SNOT 22 outcomes and improved UPSIT measurements in the CRSwNP sub group was demonstrated (125). On further sub-group analysis, the impact of smoking, age and gender were not shown to affect baseline olfaction nor olfactory function following...
surgery, suggesting that sinus disease was the most important contributor to impaired olfaction in our cohort, overshadowing other factors.

We indirectly assessed the effect of polyp size on olfaction by analyzing the correlation of UPSIT with the Lund-Mackay CT and Lund-Kennedy endoscopic scores. A negative correlation was demonstrated for all scores preoperatively (Spearman $r=-0.55$ for LM scores and $r=-0.50$ for LK scores) and thereby implying a higher or more severe pre-operative polyp score is moderately correlated with a lower olfactory performance (125).

The majority of our patients were hyposmic prior to surgery in keeping with our pre-operative UPSIT scores which averaged at 25.5 and according to the UPSIT grading system a score of 25.5 reflected a moderate to severe hyposmia (121). However given the tertiary referral nature of our practice, a more severe score may have been expected. Equally although the patient’s sense of smell improved following surgery the majority of the patients still remained hyposmic which is in keeping with current evidence.

The findings in our CRS subgroup analysis are in agreement with results from a recent study evaluating olfactory outcomes in CRSwNP and CRSsNP subgroups. A significant olfactory improvement in the CRSwNP subgroup but not in CRSsNP subgroup was demonstrated using the Sniffin Stick identification test (96). Rowe-Jones et al performed a 5 year prospective study, of whom 75% were CRSwNP, which demonstrated a significant improvement in measured olfaction at 2 years post surgery and then became non significant at 5 years (99). Interestingly Rudolf Briner et al. also demonstrated a significant improvement in olfaction following ESS which remained significant long term (94). Lund et al also demonstrated a significant improvement in olfactory function following ESS (98).

Shriever et al in 2013 looked at the effects of nasal surgery, including both sinus surgery and septal surgery, on olfaction using the 16 item odor identification ‘Sniffin’ stick test. At 3.5 months post operatively there was a significant improvement in the sinus surgery arm as opposed to a non-significant improvement in the septal surgery arm and interestingly they both became non-significant at 12 months. They also found that polyps and eosinophilia were good prognostic factors for olfactory outcome improvement and equally in the non-polyp group, the improvement was not significant (103;126). Randhawa et al uniquely demonstrated a
significant improvement in olfactory function following functional septorhinoplasty surgery using sniffin sticks at 6 months.\textsuperscript{(127)} However, Rudmik and Smith performed a literature review on the efficacy of FESS in CRS-related olfactory dysfunction and concluded the evidence supporting its efficacy is equivocal and not significant\textsuperscript{(102)}. Interestingly there is also increasing evidence supporting early surgical intervention in medically refractory CRS whereby higher post operative healthcare needs are exhibited when the delay for surgery is longer\textsuperscript{(128)}.

In our CRS subgroup study, measured UPSIT olfactory function did not change after surgery in 12.7% patients, improved after surgery in 46.0% patients and worsened in 41.3% patients. Interestingly on subjective olfaction measurements, improvement was seen in 72.5% patients, did not change in 13.7% patients and worsened in 13.7% patients after sinus surgery. Pade et al demonstrated an improvement in the sense of smell, as measured by Sniffin sticks in 23% of their post operative patients, no change was seen in 68%, and a decreased function was seen in 9% of the patients.\textsuperscript{(129)} Dealan et al. also demonstrated post-endoscopic sinus surgery olfactory improvements in 70% of their CRS population and olfaction changed for the worse in 8%.\textsuperscript{(130)}

The evaluation of our patient’s sense of smell was subjectively measured using both the UPSIT and VAS scoring methods and as a result were both open to potential patient bias and is a potential limitation of this study. The UPSIT test was chosen in this study owing to its universal track record. It’s the most validated chemosensory testing or psychophysical technique used internationally and additionally evaluates the probability of malingering in our patient group. As a forced response test from 4 potential answers, there is a 1 in 4 chance of getting a correct answer and hence a score below 8 increases the probability of malingering. The UPSIT is therefore our screening test of choice and works on a scratch and sniff principle revolving around 40 different odours. It tests cognition at the same time\textsuperscript{(121)}.

There were arguments originally pertaining to the UK validation of the original UPSIT which used names and smells which were culturally unfamiliar to the UK as well as other countries. As a result the unfamiliar smells and names of the US normative data could not be fully
transferable. This has been improved with the provision of the British version \(^{(131)}\). In this 3\textsuperscript{rd} version the American scents which were culturally unfamiliar were replaced with commonly known UK smells. The UPSIT database has also been further improved with the inclusion of an expanded normative database of 4000 men and women. This enables to provide normative data down to the 5\textsuperscript{th} percentile for each 5 year age category with a classification of hyposmic patients into mild, moderate and severe with male; mild 30-33, moderate 26-29, severe 19-25, and females; mild 31-34, mod 26-30, severe 19-25. This additional percentile information has enabled titration of the patient’s sense of smell outcomes according to their age and gender and following this their sense of smell remains unchanged following olfactory biopsy. We did not have a single case of anosmia in our 131 surgical cohort group and therefore exhibited a zero percent anosmic risk. Interestingly though the majority of our patients were hyposmic prior to surgery and although their sense of smell improved they still remained hyposmic following surgery. We also explored the usage of other chemosensory or psychophysical testing techniques such as the Sniffin stick test which we decided not to go with because at the time it was not widely used and its validation was not as universal when compared to the UPSIT. Sniffin sticks have been validated in the UK and can be additionally used to determine olfactory thresholds and discrimination \(^{(132)}\). There is also an argument for testing olfaction unilaterally as opposed to bilaterally however it was decided if a difference were to be seen it would be picked up in either case as well as the added burden and additional compliance in technique we felt there was not a need to do this \(^{(133)}\).

There is now a growing acceptance that patient’s views are essential in the delivery of high quality care. In our study, patient reported outcomes measured both symptom specific complaints using VAS scoring techniques and disease specific health related outcomes using the SNOT 22 questionnaire. Each method required the patient to measure their symptom severity and its impact on their quality of life at that specific moment in time. The same questionnaires were repeated at 6 months enabling a quantitative comparison pre and post surgery. Studies have shown that symptom severity scored at 3 months are very similar to those scored at 12 months and as a result we used 6 months as the ideal time to follow patients up and repeat measurements \(^{(134)}\). This also allowed for optimum patient capture.

A well-established approach to understanding symptom severity is with the use of a Visual Analogue Scale (VAS) which is an EPOS recommendation for determining severity of disease \(^{(74)}\). It allows patients to subjectively rate their symptoms on a 10cm linear scale,
where 0 corresponds to no symptoms and 10 is the most severe. A score of 5 is generally considered significant. However, the severity of symptoms may or may not correlate with the impact of the nasal symptoms on an individual’s quality of life, and therefore a variety of additional quality of life (QOL) measures were incorporated. Of these, the Sino-nasal Outcome Test (SNOT-22) is one of the most widely used. It is a validated 22 item health status questionnaire which was originally designed for assessment relating to sinonasal disease \(^{(120)}\). The SNOT-20 was developed from the 31-item Rhinosinusitis Outcome Measure (RSOM-31) by removing 11 items thought to be less important and forming the SNOT-20. The addition of a further two items of interest (nasal obstruction and olfaction) formed the SNOT-22, which has been demonstrated to be reliable, valid, and responsive \(^{(120)}\). It has since been validated for monitoring response to treatment for patients with chronic rhinosinusitis, septal surgery and septorhinoplasty \(^{(108)}\).

The SNOT 22 was shown to have a median value of 7 in healthy volunteers and a mean of 42 in CRS patients from a cohort of 3128 patients undergoing FESS \(^{(120)}\). In a separate study but using a similar control group the mean SNOT 22 value was 9 \(^{(135)}\). The mean post operative score of 28 at 5 years was similar to that at 3 and 12 months post operatively and represents an overall 14 point improvement \(^{(134)}\). This SNOT 22 result is similar to ours although our pre operative value of 51.9 was higher and the post operative value of 29.1 was very similar and creating a larger point improvement of 22. Three of our patients did not improve following surgery with regards to their Snot 22. Interestingly, Poetker et al, demonstrated that their patients with nasal polyps scored lower on their Snot 22 scores when compared with CRS without polyps even though they scored higher on their Lund Mackay scores \(^{(136)}\).

The NOSE questionnaire provides a further validated nasal blockage symptom specific questionnaire which specifically assesses the symptom of nasal obstruction and its consequences. This brief questionnaire consists of five questions which are used to rate the burden of nasal obstruction during the past month, with each question being scored from 0 (not a problem) to 4 (severe problem). The first question asks whether you suffer with nasal congestion or stuffiness, the second questions asks about nasal blockage or obstruction, the third question asks about trouble breathing through your nose, the fourth question asks about trouble sleeping and the fifth asks about whether you are unable to get enough air through the nose during exercise or exertion. It has been validated as a quality of life measurement in nasal septal surgery, functional septorhinoplasty and nasal valve surgery and therefore
provides another potential useful measurement in determining whether there has been a meaningful subjective change following intervention \(^{(104)}\).

Most et al categorised the nose score according to severity and divided the results into mild (5-25), moderate (30-50) and severe (55-75) or extreme (80-100) \(^{(137)}\). It was concluded that patients scoring less than 30 or 6 if not multiplied by 5 was the best indicator for not suffering with nasal blockage. Rhee et al looked at normative data using the NOSE score as well as the VAS score \(^{(138)}\). The mean asymptomatic individual NOSE and VAS scores were 15 \(^{(17)}\) and 2.1 (1.6). The mean (SD) NOSE and VAS scores for a patient with nasal obstruction were 65 (22) and 6.9 (2.3), respectively. The mean postsurgical NOSE and VAS scores were 23 (20) and 2.1 (2.2), respectively. Interestingly these results are very similar to our pre and post NOSE scores which measured 12.9 and 5.6 (before multiplying by 5).

This study was the first to evaluate the NOSE scale as an outcome measure for nasal blockage in the CRS population. The NOSE scale significantly improved in both the CRSsNP and CRSwNP subgroups following endoscopic sinus surgery, although a direct relationship with improved olfaction was not demonstrated. The NOSE questionnaire provides another validated symptom-specific quality of life questionnaire, which specifically assesses the symptom of nasal obstruction and its consequences \(^{(125)}\).

In this study the endoscopic appearances of the nasal cavity improved significantly at 6 months post operatively in both surgical arms and a difference between the two groups was not seen. A statistically significant improvement in the Lund and Kennedy score was demonstrated with a significant improvement in oedema, polyposis and discharge following endoscopic sinus surgery with and without biopsy. As a result our olfactory harvesting technique did not exhibit a significant effect on the sinus cavity appearances post operatively.

Although the surgeon reported Lund and Kennedy scores reflect a significant improvement in the biopsy and non-biopsy arms, it needs to be acknowledged that the method of nasal decongestion used in the operating theatre was not always the same as that used in clinic during the post operative evaluation and may have introduced a confounding factor particularly in the CRSsNP subgroup analysis. However, given the large sample size and the fact decongestant was always used both pre and post operatively it is unlikely to be
The key however is one of prediction and looking for the predictive factors indicating likelihood of improvement \(^{101}\). Litvack et al in their cohort of patients found age and nasal polyposis key predictive factors; 64% of men and women between the ages of 18 and 64 had olfactory dysfunction whereas 95% of patients older than 65 years had olfactory dysfunction. There was no significance found with gender. The risk of hyposmia increases with additional asthma and smoking comorbidity. Allergic rhinitis and structural defects did not affect sense of smell. The incidence of smoking among our population group was very similar to the normative UPSIT data and can therefore be reasonably extrapolated showing from our results that it does not have a significant effect on outcome. In our study we did not find that smoking affected olfactory function following surgery in either group and similarly we did not find a correlation with gender or age \(^{122}\).

Our findings do appear to agree with common opinion that hyposmia in CRS is not just a conductive problem but also a potential irreversible sensory disorder whereby even after clearing the conductive component the hyposmia remains. The sensory disorder is dependent on olfactory epithelium atrophy which is also dependent on age, disease severity and prolongation of disease and asthma. The current belief is to operate on these patients as soon as possible before the onset of irreversible disease progression \(^{128;139}\).

Importantly we have demonstrated that the patient’s sense of smell is statistically unaffected by olfactory mucosa harvesting surgery in the largest study performed so far. Our results are in keeping with other studies, albeit studies which have recruited smaller patient numbers and are also non-controlled. Say et al biopsied 31 patients and utilized the inferior segment of the superior turbinate and found that 12% of their patients had a decreased sense of smell post operatively which they concluded was not due to the harvesting procedure per se as the samples received from these patients did not contain olfactory tissue in the first place. There overall conclusion was that the sense of smell is unaffected by olfactory harvesting \(^{140}\). Equally in another study of 42 patients whereby the superior turbinate was punch-biopsied they also concluded the sense of smell was unaffected \(^{123}\).
As well as demonstrating that our olfactory harvesting technique is safe and does not affect the patient’s sense of smell we have equally highlighted that the CRSwNP subgroup represents the most surgically responsive subgroup for olfactory improvement and this will improve the informed consent process for patients. Also by improving a patient’s sense of smell following surgery we can also expect a significant improvement in the patient’s quality of life in the CRSwNP subgroup. According to our data, up to one third of the SNOT-22 variance was related to UPSIT change and not 1/22th as would be expected if it were just referring to the sense of smell. This would imply that impaired olfaction has a greater impact on quality of life than expected.

However, olfactory impairment in CRS is not just a conductive problem which would explain the lack of correlation between improved olfaction and the improved NOSE scale outcome. Conversely, we have demonstrated a significant proportion of patients whose measured and perceived sense of smell became worse after surgery, which also needs to be explained at the time of informed consent. Equally the more severe the CRS presents pre-operatively then the likelihood of olfactory dysfunction also increases. Reassuringly we did not have a single case of post operative anosmia.

One of the limitations of this study was the lack of pre-operative stratification of severity and duration of hyposmia, thus preventing a sub-group analysis of olfactory improvement taking into account these variables. In addition the UPSIT evaluation may not have been sensitive enough for olfactory loss detection as it only measures olfactory identification as opposed to the additional outcomes of olfactory threshold and discrimination unlike the Sniffin stick evaluation which measures all three. The mismatch between our measured UPSIT outcomes and what the patient perceived as measured by the VAS olfactory outcomes may relate to the sensitivity of the UPSIT evaluation. With the use of Sniffin sticks, Pade and Hummel, deemed an olfactory improvement of three or more points as a significant change and hence a similar strategy should be recommended for future UPSIT measurements\(^{(129)}\).

Another limitation of this study was the lack of a true objective olfactory outcome measurement such as event-related olfactory potentials or functional MRI scanning owing to the fact we did not have access to this technology. Equally we did not correlate the NOSE scale outcome to an objective measure of nasal airflow such as a Nasal Inspiratory Peak Flow (NIPF) which will be incorporated in future studies.
A lack of patient randomization was a limitation of this study design. At the start of this study patients were initially randomised but those allocated to olfactory harvesting were declining more than originally anticipated. Consequently a more pragmatic approach was adopted whereby all patients were invited to undergo olfactory biopsies and those who declined were placed in the control group. The reason for the initial adoption of randomisation was so as to achieve a higher evidence based impact to the study.

One of the statistical limitations of this study lay in the presumption that the power calculation for the primary hypothesis was automatically the same as that for the secondary hypothesis whereby in effect they should have been calculated separately. Reassuringly the sample size was large in both groups, as was the effect size, and therefore should not have had a significant impact. Similarly, although the data for outcomes in rhinology are typically non-parametric in presentation and the majority of the statistical methods employed in this thesis were of non-parametric design, it needs to be acknowledged that the parametric 2 way ANOVA was still employed. The 2 way ANOVA was used owing to the fact we were specifically dealing with the same patient population group with similar variance and the sample size was large. In this case the 2 way ANOVA works well with continuous data which is non-parametric, however, it is acknowledged that a non-parametric test should have also been considered. Equally to prevent confusion over patient recruitment for the CRS analysis as shown in table 2, the demographic number of CRS patients amounted to 97 and yet 113 were recruited into the CRS study. This discrepancy has arisen through the additional patients who were also recruited from the ‘other patients’ within table 2, for example, those who had fungal infection or other disease processes which may have equally presented with CRS.

Our olfactory harvesting process has undergone continual refinement so as to maximize yield rates and maintain safety and function, and as previously discussed in the interest of good engineering science, this may have confounded the results albeit most likely non-significantly as the technique was refined. In this study we had solely analysed the middle section of the superior turbinate which is predominantly supplied by the lateral olfactory bundles as they exit the nasal cavity. In order to obtain a larger sample it could be argued that the lower half of the superior turbinate could have been harvested and this would involve only one horizontal incision and a surgically much easier vertical posterior incision. The advantages of
this further refinement would be an easier surgical excision and more olfactory tissue harvested.

An area of expansion which is currently being refined involves harvesting the medial olfactory bundles which course up the septal perpendicular plate and enter the cribiform foramen as shown in figure 34. This septal mucosa area should contain a higher proportion of olfactory neurons and olfactory ensheathing cells owing to their convergence as they exit the nasal cavity via the cribiform fossa. This region which lies directly beneath the cribiform fossa can only be surgically accessed when exposing the anterior skull base and frontal sinus ostia with the assistance of image guidance techniques and is commonly visualized when performing the modified Lothrop technique. In this technique the frontal sinus floor and inter-sinus septum are drilled out and fashioned into a single opening enabling excellent exposure of the anterior skull base and olfactory prominence. The above refinement still preserves the extradural harvesting approach although current clinical studies have utilized the intradural olfactory bulb harvesting technique with very promising clinical efficacy. In this clinical study a patient’s olfactory bulb OECs were transplanted into their severed spinal cord with additional peripheral nerve grafting working on the premise a higher OEC yield is obtained from the olfactory bulb\(^{(141)}\).

Figure 34
Currently, olfaction has been shown to reflect the general neurological well-being of a person and its dysfunction could act as an early warning sign for a neurodegenerative dysfunction such as in Alzheimer’s disease. Hence, this biopsy technique could be utilized in the diagnosis and potential treatment of neurodegenerative diseases\(^{(142)}\). In addition the cause for anosmia in our rhinological practice and the reason as to why it can remain irreversible is an ongoing medical challenge; bearing in mind it also exhibits a paradoxical and exceptional regenerative ability. There is also an ongoing quest to look at new ways to treat anosmia and consequently there will be a need to perform histological analysis of olfactory epithelium in such patients so as to determine causation and help inform treatment.
CONCLUSIONS

The primary hypothesis was shown to be true and the results from this thesis demonstrate that olfactory harvesting is ‘patient safe’ and does not incur a detriment to the patient’s nasal function and specifically does not reduce the patient’s sense of smell. In this prospective controlled level 2b evidence based study we have demonstrated that patient morbidity and beneficial outcomes following harvesting human olfactory nasal mucosa during ESS is statistically the same compared to the outcomes of standard ESS without biopsy.

The secondary hypothesis which stated that the effect of sinus surgery improves olfaction in both the CRSwNP and CRSsNP subgroups has also been accepted as demonstrated by the results of this thesis. In the CRSwNP subgroup, the patient’s perceived and measured sense of smell improved significantly following ESS and represents the most surgically responsive CRS subgroup as well as the most likely subgroup to improve the patient’s quality of life following an improved post operative olfactory response. This statistically significant olfactory improvement following surgery was not demonstrated in the CRSsNP subgroup, although olfaction still improved. In addition we have uniquely demonstrated that the ‘NOSE’ patient reported outcome measure is a novel and sensitive outcome measure in the CRS population, however, it does not correlate with improved olfaction.

The future aims are to look at new ways to treat anosmia and help diagnose neurodegenerative diseases and the need to perform safely histological analysis of olfactory epithelium in such patients will help improve and inform new treatments.
Reference List


(11) Li Y, Field PM, Raisman G. Regeneration of adult rat corticospinal axons induced by


(19) Franklin RJ, Gilson JM, Franceschini IA, Barnett SC. Schwann cell-like myelination following transplantation of an olfactory bulb ensheathing cell line into areas of demyelination in the adult CNS. Glia 17, 217-224. 1996.


(40) Brann JH, Firestein SJ. A lifetime of neurogenesis in the olfactory system. Front Neurosci 26[8], 182. 2014.


(96) Lind H, Joergensen G, Lange B, Swendstrup F, Kjeldsen AD. Efficacy of ESS in

(97) Kimmelman CP. The risk to olfaction from nasal surgery. Laryngoscope. 104 (8), 981-988. 1994.


(107) Shemshadi H, Azimian M, Onsori MA, Azizababi FM. Olfactory function following


Appendix

A

Consent form

Patient information sheet

CONFIDENTIAL

Patient Information Sheet
Version 6
26th April 2015

THE SOURCE, CULTURE AND CHARACTERISATION OF HUMAN OLFACTORY ENSHEATHING CELLS OBTAINED FROM BIOPSIES OF NASAL MUCOSA.

We would like to invite you to take part in a research project. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like further information. Take time to decide whether or not you wish to take part.

Background to the study

“Olfactory ensheathing cells” are a unique group of cells that are found in the brain and nose, which can repair damaged nerve fibres. If these cells are transplanted into patients with damaged nerves or spinal cords, it may be possible to cure injuries which were previously untreatable. If we can learn how to obtain these cells from the nose and purify them in the lab, then in the future it might be possible to take cells from the nose of a patient with spinal cord injury and place them into the spine to help repair the damage and make them walk again.

However it is first necessary to find a safe and reliable way to obtain these cells from patients, and check if they can repair nerves in a similar way to previous laboratory and animal studies. We are studying a method for obtaining and growing these cells, taken from the inside of the nose, close to the area of your operation.
This work is sponsored by DePuy Spine Ltd and two charitable organisations (Spinal Research and the British Neurological Research Trust).

What will be involved?
If you agree to be in the study, your pituitary or nose operation will not be affected. The only difference is that two small biopsies (samples of tissue, about 3mm cubes) will be taken from the inside of the nose during the surgery. Any side effects are extremely unlikely.

These samples will be grown in the laboratory to obtain the olfactory ensheathing cells, and then their properties will be tested in the laboratory or in animals to check that they are safe and are capable of repairing nerves. After study, the samples will be destroyed. Your GP will be informed if you decide to take part in the study.

Do I have to take part?

Your participation in this study is entirely voluntary. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to change your mind at any time without giving a reason. If you decide not to take part, this will not affect the standard of care that you will receive.

What will happen to me if I take part?

The biopsies will be taken during the operation. This theoretically may produce a small amount of bleeding, possible infection, or problems with the sense of smell on one side. However, these side effects are very unlikely to happen, and even if they did, would probably go unnoticed. The risks posed by taking the biopsies are far smaller than the risks of the standard operation that you are having. The specimens will go to the laboratory for further study, and will be destroyed at the end of the study.

In addition, it is a requirement of the Human Tissue Authority to test donors for hepatitis B and C, syphilis, HIV and Human T-lymphotropic virus Type I and II (HTLV-1+2) before we take the specimen to our laboratory. This is to reduce infection risk and contamination in the laboratory. The results of these tests will be strictly confidential.

This study will not affect the time you spend in hospital or your after-care, and no extra out-patient reviews are required. Your sense of smell will be routinely checked at out-patient review.

What are the benefits to taking part?

There will be no direct clinical benefit for those patients undergoing biopsy during their pituitary operation. This study will be part of a bigger project to find a cure for paralysis that result from spinal cord or nerve injury. In the future it may help us to treat patients with spinal injuries.

Confidentiality
The information gathered during this study will be strictly confidential, and stored on a password-protected hospital computer for future analysis. The information stored will include patients’ names, ages, types of operation and pathology results. The samples will be anonymised, but linked to patient data and analysed by Mr David Choi. The custodian of the data will be UCLH Foundation Trust, and Mr Choi will be responsible for the security of the data. Data will not be transmitted outside the European Union and will not be stored longer than 10 years. Any publication of data will not identify you in any way.

Further questions?

If you have any further questions about the study, we would be delighted to answer them for you. Please contact Mr David Choi via the National Hospital for Neurology and Neurosurgery, Tel. 020 3448 3395.

This study has been reviewed by the National Hospital for Neurology and Neurosurgery and Institute of Neurology Joint Research Ethics Committee.

What if something goes wrong?

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone’s negligence then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you.
CONSENT FORM

THE SOURCE, CULTURE AND CHARACTERISATION OF HUMAN OLFACTORY ENSHEATHING CELLS OBTAINED FROM BIOPSIES OF NASAL MUCOSA.

Name of Researcher: ____________________________

Please initial box

1. I confirm that I have read and understand the information sheet dated 26.04.15 (Version 6) for the above study 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.

3. I understand that sections of any of my medical notes may be looked at by responsible individuals from UCLH, RNTNE Hospital, or from 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 agree to take part in the above study.

__________________________ ____________________________ ____________
Name of Patient Date Signature

__________________________ ____________________________ ____________
Name of Person taking consent Date Signature
(if different from researcher)

__________________________ ____________________________ ____________
Researcher Date Signature
THE SOURCE, CULTURE AND CHARACTERISATION OF HUMAN OLFACTORY ENSHEATHING CELLS OBTAINED FROM BIOPSIES OF NASAL MUCOSA.

Dear Dr

Your patient ………………………….. d.o.b……………………………… has agreed to take part in the above study.

Background to the study

“Olfactory ensheathing cells” are a unique group of cells that are found in the brain and nose, which can repair damaged nerve fibres. If these cells are transplanted into patients with damaged nerves or spinal cords, it may be possible to cure injuries which were previously untreatable. If we can learn how to obtain these cells from the nose and purify them in the lab, then in the future it might be possible to take cells from the nose of a patient with spinal cord injury and place them into the spine to help repair the damage and make them walk again.

However it is first necessary to find a safe and reliable way to obtain these cells from patients, and check if they can repair nerves in a similar way to previous laboratory and animal studies. We are studying a method for obtaining and growing these cells, taken from the inside of the nose, close to the pituitary area in patients who are scheduled for transphenoidal pituitary surgery or nasal endoscopic surgery.

What will be involved?

The pituitary or endoscopic operation will not be affected. The only difference is that two small biopsies of mucosa, about 3-4mm cubes, will be taken from the inside of the nose during the surgery, on the way to the pituitary gland. Any side effects are extremely unlikely. Theoretically there may be a small risk of bleeding, possible infection, or problems with the sense of smell on one side. However, these side effects are very unlikely, and even if they did occur would probably go unnoticed. The risks posed by taking the biopsies are far smaller than the risks of the pituitary or nasal operation itself.

This study will not affect the duration of the hospital admission or the after-care, and no extra out-patient reviews are required.
These samples will be cultured in the laboratory to obtain the olfactory ensheathing cells, and then their properties will be tested in the laboratory.

**What are the benefits to taking part?**

There will be no direct clinical benefit for those patients undergoing biopsy during their pituitary or nasal operation. This study will be part of a bigger project to find a cure for paralysis that results from spinal cord or nerve injury. In the future it may help us to treat patients with spinal injuries.

**Further questions?**

If you have any further questions about the study, we would be delighted to answer them for you. Please contact Mr David Choi via the National Hospital for Neurology and Neurosurgery Tel. 020 3448 3395.

This study has been reviewed by the National Hospital for Neurology and Neurosurgery and Institute of Neurology Joint Research Ethics Committee.
Papers published and accepted afforded to this MD(Res);


