The imaging tree of parathyroid diseases - Improving surgical outcomes by illuminating parathyroid glands

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CHAPTER 16

Summary and general discussion

SUMMARY AND GENERAL DISCUSSION

Parathyroid diseases are heterogeneous conditions generally characterised by the dysregulation of calcium homeostasis due to alterations in parathyroid hormone (PTH). Primary hyperparathyroidism (pHPT) is a condition in which one or more parathyroid glands produce excess PTH usually caused by an adenoma forming in one of four parathyroid glands. Less common causes are multiple adenomas, four-gland hyperplasia or a parathyroid carcinoma. In case of a single adenoma, a minimally invasive parathyroidectomy (MIP) is the preferred surgical treatment to achieve biochemical cure.[1] The success rate of the MIP as a focussed approach is comparable to the bilateral neck exploration, but surgical benefits include shorter operative time, lower complication rate, smaller incision length and saving half of the operative field as virgin territory for potential future parathyroid surgery.[2] A MIP is only feasible when imaging studies localise the abnormal gland preoperatively as the goal is to prevent a 'blind' bilateral neck exploration. The adherence to practice recommendations[1, 3] on the management and preoperative workup of patients with pHPT is low.[4, 5] With the heterogeneous management of pHPT and the variety of imaging options available, it remains a challenge to reach a consensus on the optimal preoperative workup of patients with pHPT.

Hypoparathyroidism is a parathyroid disease with insufficient PTH production, resulting in hypocalcaemia and eventually recurrent hospitalisations, depression, decreased quality of life, and even increased risk of death.[6-8] The most common cause of hypoparathyroidism is thyroidectomy caused by accidental resection or surgical damage to the parathyroid glands leading to impaired blood supply with parathyroid organ failure.[9-11] Regardless of the current efforts to reduce or prevent this outcome, such as the use of optical imaging, permanent hypoparathyroidism remains a common complication and severe condition.

Chapter 1 provides a general introduction of parathyroid diseases, the imaging modalities used in parathyroid diseases as well as an outline of the thesis.

Part I: Nuclear molecular imaging in parathyroid diseases

In the first part of this thesis, we aim to preoperatively localise parathyroid tumours using nuclear molecular imaging with significant accuracy to facilitate minimally invasive surgery. **Chapter 2** investigates the preoperative imaging workup of patients with pHPT and how they are performed within an actual clinical practice in the Netherlands. It also reviews the clinicians' adherence to pHPT treatment guidelines. Overall, we found that within this clinical practice, the preoperative imaging workup was managed in a heterogeneous manner. We illustrate that the level of compliance to preoperative imaging guidelines is suboptimal (69%) in our population. Further, MIP was correlated to whether patients' clinicians followed the imaging guidelines. Guideline adherence was also associated with a shorter length of hospital admission. The discrepancy between recommended practice and actual clinical practice in this study is striking and warrants targeted education of physicians on appropriate preoperative imaging workup for patients with pHPT. Furthermore, by studying trends in clinical practice, a more accurate estimation of adherence to guidelines can be identified and gaps in quality can be addressed.

Based on the observed non-adherence of guidelines in the management of pHPT, the treatment of pHPT could benefit from quality indicators. Quality indicators assess the quality of care to evaluate the implementation of evidence-based guideline recommendations which improve outcomes.[12-15] **Chapter 3** proposes the first pHPT quality indicators developed through a robust evidence and consensus-based methodology. Eighteen quality indicators were selected by a Canadian 9-member expert panel consisting of three endocrinologists, three otolaryngologists, and three endocrine surgeons. These indicators will be used both by individual clinicians looking for direction on evidence-based care pathways as well as heath systems to assess trends in care. Ultimately, these indicators will lead to quality improvement initiatives and more consistent effective care ensuring cost-effective, optimized outcomes for patients with pHPT.

Since the success of a MIP strongly depends on how well the adenoma is localised preoperatively, **Chapter 4** studies the diagnostic performance of ^{11}C-methionine positron emission tomography/computed tomography (PET/CT) for the detection of parathyroid adenomas in pHPT after negative first-line imaging. This retrospective analysis shows that ^{11}C-methionine PET/CT correctly localised the parathyroid adenoma in 64% of patients after previous first-line imaging failed to do so. The sensitivity of ^{11}C-methionine PET/CT for localization of parathyroid adenomas was 72%. The uptake of ^{11}C-methionine in parathyroid adenomas most likely depends on the expression and activity of amino-acid transporters such as LAT1 and secondarily on its incorporation in the protein pre-Pro-PTH.[16] ^{11}C-methionine is therefore closely related to the synthesis and excretion of PTH. Since ^{11}C-methionine PET/CT can find lesions which are invisible in most other imaging studies, more patients may qualify for MIP and therefore have shorter, cheaper, more effective surgery.

In **Chapter 5**, we optimise the imaging protocol of a ^{11}C-choline PET scan for localization of parathyroid adenomas in patients with pHPT. This was achieved by evaluating the optimal tracer uptake time, amount of radioactivity needed, and the inter-observer agreement for ^{11}C-choline PET/CT. PET scans of patients scanned for up to 60 minutes (min), were reconstructed in 10 min frames (from 10 to 60 min postinjection), mimicking varying ^{11}C-choline uptake times. Parathyroid adenoma to background contrast ratios were calculated. We found that the adenoma/background contrast ratios did not differ from the 20-30 min interval postinjection compared to the delayed intervals. This showed that the optimal time to acquire PET/CT images for parathyroid localization is 20 min after injection of ^{11}C-choline. To further build on this idea, data was reconstructed with varying scan durations (1, 2.5, 5 and 10 min) at 20-30 min postinjection, mimicking less administered radioactivity. Although there was a significant difference in (average) standardised uptake values (SUV) at shorter scan durations compared to the 10 min scan, the differences for the 2.5 and 5 min scan durations were minor, and clinically not relevant. The observed increased spread in SUV values for the 1 min scan duration was wider than for the other scan durations. To evaluate the clinical relevance of images with different quality, we performed an inter-observer study. We concluded that a 1 min scan is too short for accurate visual and quantitative interpretation of ^{11}C-choline PET/CT images. Combining the quantitative analysis and inter-observer scan interpretation results, we recommend to scan for at least 5 min. Alternatively, the radioactivity dose can be lowered by 50% while maintaining a 10 min scan duration.

After optimizing the PET/CT protocol in Chapter 5, we study the diagnostic performance of ^{11}C-choline PET/CT to detect hyperfunctioning parathyroid glands in **Chapter 6**. Due to their positive electric charge, choline-labelled radiopharmaceuticals enter the cell through a membrane transporter and accumulate in the mitochondria of parathyroid adenoma oxyphilic and chief cells. Furthermore, choline is also phosphorylated in the chief cells by choline-kinase, which is overexpressed in patients with HPT and used as a component of cell membranes.[17] We hypothesized that given choline-labelled radiopharmaceuticals can exploit a double uptake mechanism, it would be highly effective for localizing parathyroid disease.[17, 18] In this retrospective study, we included 36 patients, of which three patients were known to have Multiple Endocrine Neoplasia (MEN) syndrome. We found that the per-lesion sensitivity of ^{11}C-choline PET/CT was 97%, the positive predictive value was 95%, and the accuracy was 94% for all parathyroid lesions. Using a highly sensitive preoperative imaging technique, such as ^{11}C-choline PET/CT, might lead to more minimally invasive procedures. Correspondingly, in our cohort, the surgeon performed a successful MIP in 94% of patients.

Additionally, in **Chapter 7**, we compare the accuracy of ^{18}F-choline PET/Magnetic Resonance Imaging (MRI) to neck ultrasound and parathyroid scintigraphy (MIBI), the first-line imaging techniques, to provide more rigorous evidence on the true accuracy and added value of ^{18}F-choline PET/MRI for parathyroid adenoma localization. ^{18}F-fluorocholine, is a more widely available tracer compared to ^{11}C-choline because of its stable biodistribution and long half-life (110 min). However, the biodistributions of ^{11}C-choline and ^{18}F-fluorocholine may not be completely identical. We found that in a Canadian pHPT cohort, the sensitivity of ^{18}F-choline PET/MRI was superior to that of ultrasound and MIBI combined. In the 19 patients in whom both US and MIBI were negative, PET/MRI correctly identified the parathyroid adenoma in 13 patients (68%). An interesting finding in our study was that MRI also identified thyroid nodules needing additional subsequent investigation or therapy. ^{18}F-choline PET/MRI could become a valuable preoperative localization study given its superior performance in localizing parathyroid adenomas and accuracy in thyroid nodule detection. A single PET/MRI could both provide the localization of parathyroid disease from an anatomical and functional perspective as well as assessment of thyroid nodules, something that no other modality used for these patients can do. This may change the approach to surgical planning in patients with pHPT.

There has been a surge in studies using PET and CT scans to localise parathyroid adenomas preoperatively. Both ^{11}C-methionine PET/CT, ^{18}F/^{11}C-choline PET/CT and four-dimensional CT (4D-CT) are frequently employed worldwide in patients with pHPT.[19-26] However, no studies are available comparing these three techniques directly. Therefore, it remains unclear which of the possible imaging modalities is superior and should be performed as second-line scan after negative first-line imaging. We prospectively compare the sensitivity of ^{11}C-methionine PET/CT, ^{11}C-choline PET/CT and 4D-CT for detection of parathyroid adenomas to explore in which order to use each of these imaging techniques as second-line scans in the setting of pHPT in **Chapter 8**. In this interim report, we found that ^{11}C-choline PET/CT is superior to ^{11}C-methionine PET/CT and 4D-CT in localizing parathyroid adenomas, allowing localization of parathyroid adenomas or hyperplasia in 85% of parathyroid lesions. Given choline-labelled radiopharmaceuticals can exploit a double uptake mechanism could prove advantageous of choline PET/CT compared to methionine PET/CT.[17, 18] The choice of ^{11}C-choline rather than 4D-CT, next to its superior diagnostic effectiveness, is also supported by the consideration that 4D-CT involves a higher radiation exposure than ^{11}C-choline PET/CT.[27, 28] Interestingly, in this study, in all 12 patients in whom ^{18}F-fluorocholine PET/CT was negative or inconclusive, ^{11}C-choline PET/CT could identify a lesion suspicious of a hyperfunctioning parathyroid gland. Therefore, in this study, ^{11}C-choline PET/CT seemed superior to ^{18}F-choline PET/CT for localization of parathyroid lesions. The cause of this finding remains speculative. We hypothesize that is mainly related to differences in scan protocols. Also, differences in biodistribution of the tracers cannot be ruled out. Ultimately, we advocate for the performance of ^{11}C-choline PET/CT after negative first-line imaging in patients with pHPT.

Organoids are 3D structures that closely recapitulate a specific tissue architecture and cellular composition and are developed from stem cells.[29] Organoid models are useful for studying tumour behaviour and assessing drug responses and provide a platform for long-term *in vitro* experimentation.[30-35] In addition, they can be used for further understanding and development of effective parathyroid tracers for diagnostic imaging. In **Chapter 9**, we develop a patient-derived parathyroid organoid model for tracer and drug-screening applications. To this aim, patient-derived hyperplastic parathyroid tissue was dispersed, and parathyroid organoids were cultured, showing self-renewal potential suggesting the presence of stem cells. RNA-sequencing confirms that parathyroid organoids phenocopy hyperplastic parathyroid tissue. Additionally, we illustrate clinically relevant feedback mechanisms (increased and decreased PTH secretion) of human parathyroid organoids in response to changes in calcium concentration and PTH lowering drugs. Furthermore, we found specific parathyroid-targeted tracer uptake (^{11}C-methionine and MIBI) in our organoids, demonstrating that these organoids can model human parathyroid functionality. This parathyroid culture will be able to provide a novel *in vitro* model to perform physiology studies and discover potential novel therapeutic targets, opening opportunities for future tracer testing and drug screening of the parathyroid glands.

Part II: Optical and optoacoustic imaging to support preservation of parathyroid glands

In the second part of this thesis, we discuss the application of optical and optoacoustic imaging to support preservation of parathyroid glands and prevent hypoparathyroidism. Since the reported incidence rate of hypoparathyroidism in the literature is highly variable, **Chapter 10** evaluates the incidence of postoperative, persistent hypoparathyroidism after total thyroidectomy in patients referred to university hospital centres in the Netherlands. In this cohort study, the risk of persistent hypoparathyroidism after total or completion thyroidectomy was 15%. The incidence found here compares unfavourably with data from high-volume single centres that report incidences of less than 5%.[36-38] However, it is similar to data from a multicentre study, a survey study and a nationwide study (incidence of 13% - 17%).[39-41] These numbers reflect the discrepancy in incidence rates between single-centre studies and national registries, which may be explained by different definitions for hypoparathyroidism. We demonstrate that the rate of persistent hypoparathyroidism varied between 14.5% to 28.5% depending on the definition used. These data provide a better understanding of the magnitude of this postoperative complication and highlight more realistic risks for patients undergoing extensive and complex thyroid surgery. Efforts should be made to reduce this complication rate and use uniform definitions for classification of disease and institute evidence-based treatment guidelines to find ideal treatment algorithms.

To reduce hypoparathyroidism, optical imaging techniques have been employed to identify the parathyroid glands and to estimate whether they have been spared during thyroidectomy. Optical imaging consists of (intraoperative) modalities that use the visible to near-infrared (NIR) spectrum of light.[42] The three optical imaging techniques most commonly used in parathyroid glands include parathyroid autofluorescence, indocyanine green (ICG) angiography, and laser speckle contrast imaging. Autofluorescence is employed for parathyroid identification, while ICG-angiography and LSCI are used for assessing the perfusion status of the parathyroid glands. Recent reviews on optical imaging of parathyroid glands in thyroid surgery reported varying effectiveness in predicting postoperative outcomes.[43, 44] To examine this, **Chapter 11** outlines an overview of the optical imaging techniques used to identify and preserve the parathyroid glands, explicitly focusing on the methodology used in the literature. We found that consensus in the applied protocols for the different imaging techniques is lacking and is non-uniform in areas of patient selection, imaging protocol and analysis. Although the field of image-guided surgery for localization and preservation of the parathyroid glands has shown promising results, standardization of the methodology is critical to optimize its use in clinical practice. Specifically, quantification models may provide a more objective interpretation of perfusion imaging and lead to universal application for optimizing patient outcome.

The optical imaging technique ICG-angiography allows observers to characterise tissues visually by utilising NIR light from the administered ICG fluorophore. Since ICG is bound to plasma proteins, ICG-angiography visualises blood flow, making it possible to assess the parathyroid perfusion status non-invasively. Most uses of ICG for tissue perfusion intraoperatively involve subjective review of the light patterns and real-time surgical opinion as to whether a specific tissue is adequately perfused or not. To address the problem of standardization and quantification in parathyroid optical imaging identified in Chapter 11, in **Chapter 12**, we develop a reproducible and generalizable Workflow model of ICG-angiography integrating Standardization and Quantification (WISQ). This model can be applied objectively to support the surgeon in deciding if a parathyroid gland is well perfused or if autotransplantation is needed thereby reducing the incidence of postoperative hypoparathyroidism. Given several factors influence the inter-operative signal intensity of ICG, it is understandable that perfusion cannot be determined by visual interpretation of the individual surgeon based on the absolute fluorescence intensity. As in other fields of diagnostic angiography, WISQ determines perfusion by the dynamics of an inflow and outflow graph, characterised by the shape of the curve. In this two-centre, proof-of-concept study of 10 patients, WISQ was able to standardise and quantify ICG-angiography and provide a robust and reproducible perfusion curve analysis. A low ingress slope of the perfusion curve combined with a compromised egress slope was indicative of hypoparathyroidism in 100% of the cases. Although WISQ needs prospective validation in larger series, applying WISQ is the ultimate goal for standardizing the use of ICG in thyroid surgery. WISQ aims to support further clinical decision-making to predict and ultimately prevent postoperative hypoparathyroidism. WISQ is demonstrated in a video publication in **Chapter 13**.

A further method to reduce postoperative hypoparathyroidism is to ensure surgical management is offered only to those who will benefit. The de-escalation of thyroid nodule surgery is a key component of minimizing this and other surgical complications to optimize patient outcome. Therefore, in **Chapter 14**, we study if multispectral optoacoustic tomography (MSOT) could improve cancer risk stratification by improving the accuracy of nodule assessment, eventually reducing overtreatment (diagnostic thyroidectomy) and morbidity. MSOT enables non-invasive molecular imaging at depths of several centimeters. By detecting ultrasound waves induced by pulsed laser light illumination, termed "optoacoustic waves", MSOT can distinguish and quantify different intrinsic tissue chromophores.[45] In this pilot study, we show that the use of data analytics methods for clinical MSOT imaging provides superior image quality. Combining rich spectral contrast and high resolution, characterization of spectral features in thyroid nodules is optimized. We examined 38 thyroid nodules and linked observed spectral features on MSOT to pathology data. We identify spectral features that may be associated with malignancy, such as abundance of microvascularity in thyroid cancer nodules, and visualize extrathyroidal extension in the surrounding muscle. Unfortunately, we were unable to quantitatively differentiate malignant from benign thyroid nodules, as is suggested in the initial thyroid MSOT literature.[46-48] To understand this finding, we present possible causes for erroneous quantification of biomarker presence, such as image artefacts, spectral colouring, spectral cross talk and masking by strong absorbers. We emphasise that visual inspection of MSOT images can lead to incorrect interpretation. Inferring that there is a presence of intrinsic biomarkers based on the mean absorption spectrum in a defined region of interest, might contradict biological reality. Therefore, we recommend that investigating spectral patterns of malignancy within a region of interest rather than quantifying the mean absorption spectrum within a region of interest could improve thyroid nodule characterization. This pilot study was the first step in identifying possible spectral features that could be used for characterization of thyroid nodules. We acquired the highest quality optoacoustic images of thyroid nodules published to our knowledge, pushing the utility of MSOT in the understanding and diagnosis of thyroid nodules in the future.

Similar to nuclear imaging, fundamental challenges lay ahead for optical and optoacoustic imaging. Applying proven strategies from the field of nuclear imaging will contribute to overcoming those hurdles. The optical field should benefit from the knowledge gained in nuclear tracer chemistry, standardisation and quantification and the established pathway to bring imaging techniques into the standard of care. After having experienced these challenges that lay ahead for optical and optoacoustic imaging ourselves throughout this thesis, in **Chapter 15**, we appeal to all colleagues in our communities, including surgeons and imaging specialists, to join forces as a clinical molecular imaging community and share our lessons learned, irrespective of the applied imaging modality. We aim for our communities to embrace optical imaging as a growing branch on the clinical molecular imaging tree and a global opportunity to enrich our molecular armamentarium for the benefit of the patient.

FUTURE PERSPECTIVES

Primary hyperparathyroidism

As shown in this thesis, the use of preoperative (nuclear) imaging can aid surgical decision-making in patients undergoing parathyroidectomy. Standard first-line imaging localization worldwide has historically consisted of cervical ultrasonography (US) and sestamibi scan.[1, 49, 50] However, first-line imaging has only moderate sensitivity forcing many patients to undergo a bilateral neck exploration to ensure cure.[49, 51] A variety of second-line imaging techniques are currently available when first-line imaging is negative. Despite the growing number of scientific articles published on this topic, there is currently no consensus as to a second line imaging modality for patient with non-localized parathyroid disease preoperatively.

This thesis compares multiple second-line scans for detection of parathyroid adenomas to explore which modality when at what point in the patient care pathway should be used. To determine which second-line imaging techniques are most beneficial, studies into the cost-effectiveness are needed. Feasibility of these techniques as first-line parathyroid localization tools must be explored specifically. These studies should also take multimodal imaging techniques into consideration either in tandem or sequentially. Additionally, efforts should be made to design robust registries which capture imaging and clinical outcomes in addition to administrative data, further serving to highlight important outcomes and provide guidance for health systems to incentivise quality.

Hypoparathyroidism

As shown in this thesis, hypoparathyroidism is a common complication of thyroid surgery and is frequently underreported in literature. The incidence of persistent hypoparathyroidism in the Netherlands is 15%, mandating lifelong calcium and vitamin D supplementation. Despite hypoparathyroidism being such a common complication, we lack uniformity in defining who has this disease and how they should be treated. We emphasise the need to develop national uniform diagnostic and treatment guidelines to enable informative research studies. An international consensus for the definition of persistent hypoparathyroidism after thyroid surgery and the development of reliable registration systems can serve national and international benchmarking purposes that aim for improved quality for patients who need thyroid surgery.

In this thesis, we explored the application of optical and optoacoustic imaging in phase I studies to influence surgical decision-making and support the preservation of parathyroid glands to prevent hypoparathyroidism. To determine the benefit for patients in subsequent phase II and III studies, standardization of imaging methodology and outcome parameters is imperative. Fluorescence intensities are influenced by multiple factors, such as camera to woundbed distance, ambient lighting and dynamic range of the fluorescence camera. Therefore, for future studies, (inter)national imaging protocols should be specified, with standardisation of camera settings, working distance, the ambient light, the angle of illumination, the indocyanine green (ICG) dose and dose infusion protocol, and with harmonisation across different camera types. Furthermore, if optical imaging were to be implemented into the standard of care, it needs an established uniform translational pathway. Optical-imaging-adopting clinicians need partners who can support them with a proven platform and longstanding experience on how to adopt a sound and reliable methodological development in clinical adaptation. The pathway developed to bring nuclear medicine and molecular imaging into the standard of care may provide the needed roadmap for advanced clinical translation of optical imaging. The optical imaging community should benefit from the knowledge gained in nuclear medicine standardisation, harmonisation and quantification. Only by collaboration, can we bring this field forward.

Within thyroid surgery, standardization and quantification are imperative for interpretation of ICG-angiography in the assessment of parathyroid gland viability. As shown in this thesis, we found that the process of inflow and outflow of ICG was fundamentally distinctive in poorly-perfused parathyroid glands. Our data showed a lower inflow slope and a more compromised outflow slope of the perfusion curve compared to well-perfused parathyroid glands. An approach combining biophysics-inspired modelling and machine learning (ML) can analyse intraoperative fluorescence intensities over time, providing real-time information of the parathyroid perfusion status with high specificity. To improve surgical decision-making, the incorporation of artificial intelligence (AI), which is not described in this thesis, could be of additional value in the quantitative evaluation of ICG-angiography. AI leverages computers and machines to mimic the problem-solving and decision-making capabilities of the human mind. ML uses neural networks, statistics, operations research, and physics methods to find hidden insights in data without being explicitly programmed where to look or what to conclude. It has already been shown that ML can yield increased sensitivity of medical imaging.[52-54] We hypothesise that an ML approach could distinguish poorly-perfused from well-perfused parathyroid glands with intraoperative use of ICG-angiography. This ML model could be used to support the surgeon in identifying compromised parathyroid glands and help decide if autotransplantation during total thyroidectomy is warranted, mitigating postoperative hypoparathyroidism.

ML could also be of use in the application of multispectral optoacoustic tomography (MSOT). Since hypoparathyroidism may be prevented by de-escalation of thyroid surgery, implying personalized management of patients with thyroid nodules, in this thesis we showed that the use of data analytics methods for clinical MSOT imaging allowed for characterization of spectral features in thyroid nodules. We hypothesize that the application of ML for identifying features predictive of malignancy on MSOT may increase the accuracy of this modality. ML may also diminish artefacts in MSOT images which may be a main cause of an incorrect diagnosis in clinical applications. When optimized, MSOT may combat overtreatment of patients with thyroid nodules and ultimately minimize postoperative morbidity leading to a higher quality of life for patients with thyroid nodules.

With rapid advances in imaging techniques, clinicians and scientists now collect complex data in ever-increasing amounts. To support sharing of global knowledge on imaging processing and create a big data platform for, amongst others, developing ML models, we advocate for a public, (inter)national imaging data cloud. Large organizations are already looking into solving this problem but we believe the leadership of both regional jurisdictions collaborating with professional organizations is most important for success.

Other optical imaging techniques for preserving parathyroid glands should also be considered, specifically laser speckle contrast imaging (LSCI). This technique produces a random interference pattern using laser light, forming the so-called speckle pattern. Movement of particles in the tissue of interest causes a change in the speckle pattern, resulting in blurring of the speckle images. This blurring can be correlated to blood flow as the fluctuations are caused by the movement of red blood cells. This pattern change allows LSCI to visualise and quantify blood flow in real-time without needing a fluorescent dye.[55] It has been shown that LSCI can predict postoperative hypoparathyroidism.[56] The advantage of LSCI is that it can provide continuous assessment of vessel perfusion during surgical procedures given it does not require a contrast agent. Furthermore, LSCI provides a more precise measure of the flow of a particular area, compared to ICG is innately measuring the blood volume.[57] In ICG-angiography, larger vessels (which carry more plasma) produce a stronger signal due to the increased amount of ICG fluorescent dye, highlighting this technique visualizes blood volume rather than blood flow. Still, an advantage of ICG is that it can identify feeding as well as draining vessels where LSCI can not. Therefore, LSCI and ICG-angiography provide complementary information about vasculature and potentially may be used together for optimal understanding of parathyroid viability.

ICG perfusion curves are depended on surgeons localizing a parathyroid gland correctly. Given the difficulty of the anatomy, specifically in patients with advanced nodal disease from thyroid cancer, this could be further complicated. A specific parathyroid tracer could therefore be of value to localize the parathyroid glands intraoperatively with high specificity. Moreover, multispectral imaging, i.e., the combination of a targeted parathyroid gland fluorescent tracer for identifying the parathyroid glands and the subsequent evaluation of its perfusion with ICG-angiography and/or LSCI, might diminish the occurrence of hypoparathyroidism. Multispectral fluorescence imaging requires acquisition of images at several key wavelengths, and in this case, would require two fluorescent tracers at two different wavelengths to separate them from one another.

Already, imaging agents are both fluorescently and nuclear labelled to perform both PET and fluorescence imaging.[58-60] The design of such hybrid tracers can combine desirable features of two different imaging modalities and potentially improve quantification and understanding of the underlying biological targets.[61] A parathyroid specific tracer would enable surgeons to preoperatively localise the (overactive) parathyroid gland (with a PET scan) as well as guide intraoperative surgical decision-making by identifying and sparing them intraoperatively. For the clinical development of new imaging tracers, an established appropriate preclinical model for testing is required.[62] In this scenario, our patient-derived parathyroid organoid culture methodology could be of benefit, creating opportunities for future tracer testing. Moreover, these organoids also hold potential applications in new drug discovery.

Unfortunately, intraoperative trauma to the parathyroid glands may be common, and autotransplantation does not always save the parathyroid gland function. Consequently, patients develop persistent hypoparathyroidism leading to recurrent hospitalisations, depression, decreased quality of life, and even increased risk of death.[6-8] We hope that our cultured parathyroid organoids may help provide stem cells for allogenic transplantation to alleviate suffering in patients with hypoparathyroidism. For this application, we first suggest a comparison between healthy parathyroid tissue and hyperplastic parathyroid tissue is important. We hypothesize that by transplantation of parathyroid organoids in a hypoparathyroidism animal model (rats with a total parathyroidectomy[63]), we will show that parathyroid glands may be restored in its functionality to some extent.

OVERALL CONCLUSION

This thesis illustrates that the application of nuclear, optical and optoacoustic imaging of parathyroid glands may profoundly alter surgical decision-making in patients with parathyroid diseases. The use of a standardised nuclear imaging workup enables preoperative localization of parathyroid diseases with high degree of accuracy, thereby facilitating minimal invasive surgery and lower complication rates in patients with primary hyperparathyroidism. Moreover, we show the potential of standardised and quantitative optical and optoacoustic imaging in thyroid surgery patients to support clinical decision-making and ultimately prevent parathyroid function impairment. To incorporate these imaging techniques into the standard of care, we need to create international consortiums to standardise evidence-based treatment guidelines which consist of recommendations for imaging methodology of parathyroid glands and outcome parameters. Eventually, using the imaging tree of parathyroid diseases to illuminate parathyroid glands may ultimately guide surgical decision-making and improve surgical outcomes.

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