Pituitary Tumors

Epidemiology and the Diagnostic Value of ^{68}Ga-DOTATOC-PET

Axel Tjörnstrand

Department of Internal Medicine and Clinical Nutrition
Institute of Medicine, Sahlgrenska Academy
University of Gothenburg
Sweden

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Sweden

Cover illustration: sagittal PET-MRI image of a patient with thyrotroph macroadenoma, all necessary permissions obtained.

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axel.tjornstrand@vgregion.se

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To seek the light of truth, while truth the while
Doth falsely blind the eyesight of his look.
Light seeking light doth light of light beguile;
So, ere you find where light in darkness lies,
Your light grows dark by losing your eyes.

- Berowne, William Shakespeare's Love's Labor's Lost

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ABSTRACT

Pituitary neuroendocrine tumors (PitNETs) are considered common in the general population. However benign in most cases, these tumors can evoke dramatic morbidity in patients with aggressive tumor growth and/or excess hormone secretion. Both the primary diagnostic and the post-operative imaging assessment face difficult challenges with today diagnostic methods. Also, modern epidemiological data is scarce in publications regarding these tumors and needs to be updated. The overall aim of this thesis was to study regional PitNET incidence and epidemiological data, and to describe the pre- and post-operative diagnostic properties of ^{68}Ga-DOTATOC-PET.

Paper I was an observational cohort study presenting data from 592 adult PitNET patients living in the Västra Götaland Region diagnosed between 2001-2011. Papers II-III were prospective, case-controls studies evaluating ^{68}Ga-DOTATOC tracer uptake in patients with PitNETs and healthy control subjects. Uptake data was analyzed in relation to immunohistochemical data of cell type and somatostatin receptor (SSTR) expression.
In Paper I, we found the standardized incidence rate (SIR) of PitNETs during the study period was 3.9 cases/100,000/year, which was higher than previously reported from the same region. Non-functioning PitNETs were the most commonly diagnosed (54% of tumors) followed by lactotroph (32%), somatotroph (9%), corticotroph (4%, and thyrotroph (0.7%) tumors. ^{68}Ga-DOTATOC uptake correlated with SSTR2 expression. Tumor uptake was higher in thyrotroph tumors, lower in in gonadotroph and corticotroph tumors in comparison to the uptake in normal pituitary tissue. In tumors with high preoperative tracer uptake (>13.8 SUVmax) the tracer was able to predict clinical remission if post-operative uptake was reduced under 60% of the pre-op scan.

In conclusion, PitNET SIR was higher compared to previously reported data from the same region before MRI became clinical routine. ^{68}Ga-DOTATOC can be used to differentiate corticotroph, gonadotroph, and thyrotroph tumors from normal pituitary gland. Post-operative scans suggest clinical value in predicting remission but need to be studied further.

Keywords: positron emission tomography, ^{68}Ga-DOTATOC, pituitary tumors, SSTR imaging, pituitary neuroendocrine tumor, PitNET

SAMMANFATTNING PÅ SVENSKA

Bakgrund: Tumörer i hypofysen är vanligt förekommande. Vanligen rör det sig om små godartade förändringar som inte ger några symtom. En mindre andel av tumörerna kan dock växa okontrollerat och/eller utsöndra hormoner som kan leda till omfattande systemiska förändringar hos patienten. Standardutredning av hypofystumörer sker med hormonell analys samt med magnetkamera (MR). Epidemiologisk data om hypofystumörer är begränsad, i synnerhet från modern tid då MR blivit klinisk rutin vid utredning. Inneliggande utredning blir ofta aktuellt i oklara fall och i vissa fall krävs ytterligare utredning med invasiva metoder. Stora hypofystumörer och tumörer med hormonell överproduktion kräver kirurgisk behandling. Postoperativt finns det vissa problem med nuvarande metoder att utvärdera resultatet. Postoperativa förändringar i hypofysområdet och kvarvarande tumörvävnad kan vara svåra att skilja från varandra. Både pre- och post-operativt är behovet idag stort av en effektiv bilddiagnostik som kan avspegla funktionen i vävnaden och därmed ge ett avsevärt bättre beslutsstöd för kliniker.

Mål: Att studera regional epidemiologisk data hos personer med hypofystumörer. Att utvärdera om ^{68}Ga-DOTATOC-PET kan förbättra primärdiagnostiken och den postoperativa utvärderingen hos patienter med hypofystumör.

Metoder: Data hämtas från det svenska hypofysregistret. Vuxna patienter i Västra Götalandsregionen, diagnostiserade med hypofystumör mellan 2001-2011, inkluderas i analysen. Upptag av ^{68}Ga-DOTATOC i tumörvävnad mäts innan och sex månader efter operation hos patienter med hypofystumörer. En hypofysfrisk kontrollgrupp används som jämförelse. Analys av vävnadsprover som beskriver celltyp och uttrycket av somatostatinreceptorer kompletterar den bildmässiga datan som genereras av PET och MR kamrerna.

Resultat: i VGR insjuknade 3.9 personer per 100,000 invånare per år med hypofystumör. Icke hormonproducerande tumörer var den vanligaste tumörtypen och stod för 54% av tumörerna. Övriga tumörgrupper var laktotrofa (32%), somatotrofa (9%), corticotrofa (4%) och thyrotrofa tumörer (0.7%) Upptaget av tracern ^{68}Ga-DOTATOC var efter 45 minuter signifikant högre i thyrotrofa tumörer jämfört med upptaget i normal hypofys. I både corticotrofa och gonadotrofa tumörer var upptaget lägre jämfört med normal hypofys. För tumörer med ett högt tracerupptag, visade det sig att ett klart sänkt upptag vid den postoperativa utredningen kunde förutsäga klinisk förbättring.

Konklusion: Incidensen var högre jämfört med tidigare studier innan MR blev klinisk rutin. ^{68}Ga-DOTATOC kan användas för att skilja mellan gonadotrofa, corticotrofa, thyrotrofa tumörer från normal hypofys.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. **The incidence rate of pituitary adenomas in western Sweden for the period 2001–2011**
Tjörnstrand A, Gunnarsson K, Evert M, Holm E, Ragnarsson O, Rosén T, Filipsson Nyström H.
European Journal of Endocrinology 2014;171:519-526.

II. **Lower ^{68}Ga-DOTATOC uptake in nonfunctioning pituitary neuroendocrine tumours compared to normal pituitary gland - a proof-of-concept study**
Tjörnstrand A, Casar-Borota O, Heurling K, Schöll M, Gjertsson P, Himmelman J, Itsenko O, Ragnarsson O, Filipsson Nyström H.
Clinical Endocrinology 2020;92:222-231.

III. **Pre- and postoperative ^{68}Ga-DOTATOC positron emission tomography for hormone-secreting pituitary neuroendocrine tumors**
Tjörnstrand A, Casar-Borota O, Heurling K, Schöll M, Gjertsson P, Ragnarsson O, Filipsson Nyström H
Submitted

CONTENTS

ABBREVIATIONS

ADH	Antidiuretic hormone
ACTH	Adrenocorticotropic hormone
CI	Confidence interval
CSF	Cerebrospinal fluid
CT	Computed tomography
DRG	Diagnosis-related group
E_{mean}	Mean energy
FDG	^{18}F-flourodeoxyglucose
FET	^{18}F-fluoroethyltyrosine
FSH	Follicle-stimulating hormone
GH	Growth hormone
ICD	International Classification of Diseases
IC_{50}	Half maximum inhibitory concentration
IGF-1	Insulin-like growth factor 1
IQR	Interquartile range
IRS	Immunoreactive score
LH	Luteinizing hormone
MET	^{11}C-methionine
MRI	Magnetic resonance imaging
MRS	Magnetospectroscopy
NET	Neuroendocrine tumor
NF	Non-functioning
NF-PitNET	Non-functioning pituitary neuroendocrine tumor
OT	Oxytocin
PET	Positron emission tomography
PitNET	Pituitary neuroendocrine tumor

PRL	Prolactin
R_{mean}	Mean range in water
ROC	Receiver operating characteristic
SF-1	Steroidogenic factor 1
SIR	Standardized incidence rate
SPR	Swedish pituitary registry
SST	Somatostatin
SSTR	Somatostatin receptor
SUV	Standardized uptake ratio
SUV_{max}	Maximum standardized uptake ratio
T1W	T1-weighted
T2W	T2-weighted
T-Pit	T-box transcription factor
TSH	Thyroid-stimulating hormone
VGR	Västra Götaland region
VOI	Volume of interest
W	Weighted
WHO	World Health Organization

DEFINITIONS IN SHORT

Pituitary tumor

Pituitary tumor is a heterogenous term including all space-occupying lesions that may arise from structures comprising the sellar region, e.g. benign cysts, germ cell tumors, lymphomas, meningiomas, metastases, and vascular, granulomatous, and inflammatory lesions. Tumors arising from hormone-secreting cells in the anterior part of the pituitary gland, pituitary adenomas, constitute the vast majority (~85%) of these tumors. Other types of tumors each constitute only a few percent. Since pituitary adenomas constitute the absolute majority of this group, the terms pituitary tumors and pituitary adenomas are often used interchangeably in clinical jargon.

Pituitary adenoma

Pituitary adenoma is a neoplasm composed of hormone-producing cells in the anterior (adenohypophyseal) part of the pituitary gland. These tumors are classically considered benign but may present various behaviors that would normally define aggressive neoplasms. Invasive growth into adjacent anatomical structures and recurrent tumor growth after surgical treatment are frequently seen. Malignant behaviors such as metastases are uncommon but, when they are occasionally reported, they are then classified as pituitary carcinomas by classic definition.

Pituitary neuroendocrine tumor (PitNET)

Revised nomenclature, introduced 2017, had the aim of broadening terminology from a benign connotation and to better reflect the wider range of biologic behaviors from these tumors.[1] The benign implication of the term 'adenoma' does not accurately reflect the plurality of clinical manifestations in

these tumors. Invasion and regrowth of proliferative lesions and persistence of hormone hypersecretion cause significant morbidity and mortality. The change in nomenclature might seem to be of purely academic interest but it may have thorough on-going clinical consequences. Tumors classified as adenomas, and thus considered as benign lesions, are sometimes not reported in cancer registries. In nations with privately organized healthcare systems, patients could be denied access to health insurance coverage for therapies that would otherwise be provided for 'cancers'. The revised term also attempts to better illustrate the common linage with other somatostatin receptor-expressing tumors arising from cells in the neuroendocrine system. Examples of such neuroendocrine tumors are carcinoid tumors in the gastrointestinal tract and the lungs; insulinomas and gastrinomas in the pancreas; medullary carcinoma in the thyroid gland; and pheochromocytoma in the adrenal glands. In support of these rationales, this thesis will use this up-to-date nomenclature when referring to tumors from adenohypophyseal cells.

1 INTRODUCTION

1.1 PITUITARY GLAND

The pituitary gland is central in the body's hormonal regulatory system. It is located in an anatomical depression in the sphenoid bone, called the sella turcica. It is surrounded by important structures, such as the cavernous sinuses laterally, which contain central venous vessels as well as the internal carotid arteries and cranial nerves. Superiorly to the pituitary lies the optic nerves and the optic chiasma.

Anatomically, the pituitary gland is composed of three parts, the anterior, the posterior, and the intermedia parts (Figure 1). The posterior part, also called the neurohypophysis, is functionally connected to the hypothalamus through the pituitary stalk (Figure 2A). The hormones secreted here, antidiuretic

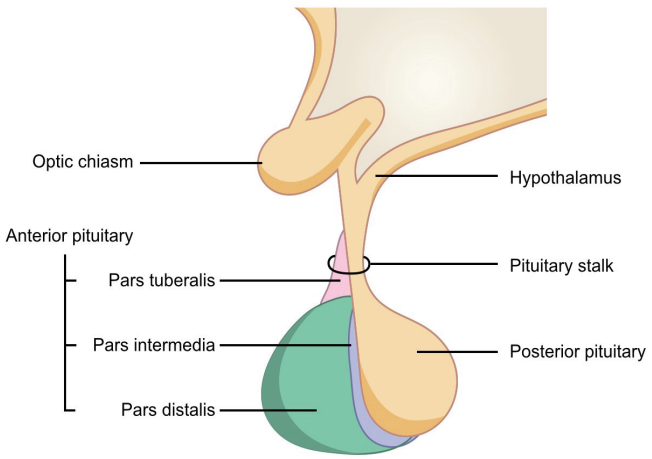

Figure 1. *The pituitary gland and its structural parts. The posterior lobe, neurohypophysis, is functionally and anatomically connected to the hypothalamus via the pituitary stalk. The anterior lobe, adenohypophysis, is where PitNETs arise. The intermedia part is mostly rudimentary in humans. Courtesy of Assoc Prof Frank Gaillard, Radiopaedia.org, rID: 75156.*

hormone (ADH) and oxytocin (OT), are produced as granules in the hypothalamus and transported via extended nerve cells for future secretion by cells in the neurohypophysis. The intermediate part is involved in melanocyte-stimulating hormone production in infants, but is mostly rudimentary in adult humans. The anterior part, the adenohypophysis, is composed of hormone-producing cells that regulate a vast array of essential physical functions. Lactotroph cells produce prolactin (PRL), inducing lactation and regulating fertility; somatotroph cells produce growth hormone (GH), promoting growth of corporal tissues; corticotroph cells produce adrenocorticotrophic hormone (ACTH), regulating the stress response from the adrenal glands; thyrotroph cells produce thyroid-stimulating hormone (TSH), regulating metabolic activity via thyroid hormones; and gonadotroph cells produce luteinizing hormone (LH) and follicle-stimulating hormone (FSH), both essential for gonad functionality and sexual development.

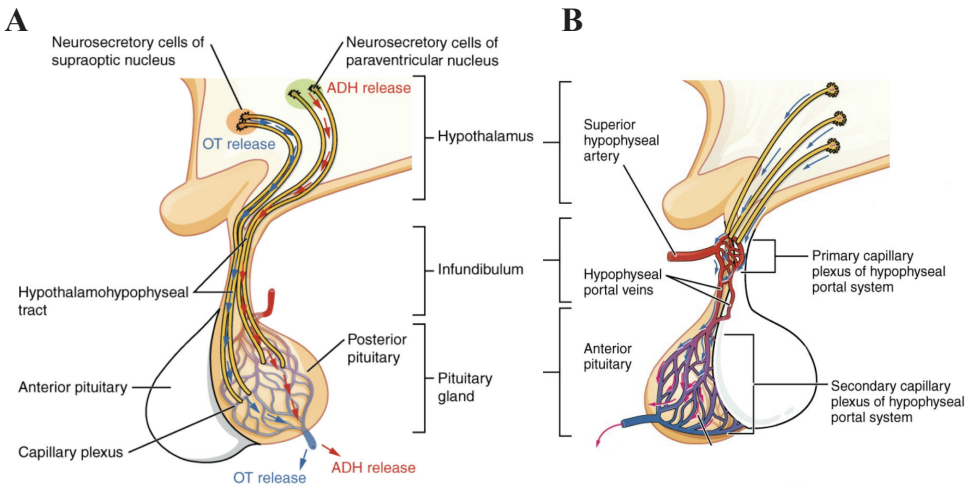

Figure 2. *The regulatory system for the two functional parts of the pituitary gland.* ***A.*** *The posterior lobe, connected directly by nerve fibers from the hypothalamus, that release granulated hormones produced by neurons in the hypothalamus.* ***B.*** *The anterior lobe and the portal venous system that functionally connect the hypothalamus with the anterior lobe. Courtesy of OpenStax College, licensed with CC BY-SA 3.0.*

The production of the adenohypophyseal hormones is regulated by release of precursor hormones secreted by the hypothalamus. These hormones are endocrinologically released in a portal venous system connecting the hypothalamus and the adenohypophysis (Figure 2B). The hypothalamus is, in turn, under regulatory control by a negative feedback loop of hormones secreted by the anterior pituitary and/or the hormones produced by their target organs. That means the hormones produced by the adenohypophysis or their target organs will act as an inhibiting factor for production and secretion in the hypothalamus.

The hypothalamus also regulates hormone levels by a hormone called somatostatin (SST), which acts by binding to five types of somatostatin receptors (SSTRs), SSTR1-5. Endogenous SST binds with high affinity to each of these receptors and the activation induces decreased angiogenesis via SSTR1, decreased hormone secretion by SSTR2 and SSTR5, increased apoptosis by SSTR3, and cell cycle arrest by SSTR4.[2-4]

All space-occupying lesions arising in the sella turcica are defined as pituitary tumors. Pituitary neuroendocrine tumors (PitNETs) constitute the vast majority of these tumor types, comprising approximately 85% of all tumor findings in the sellar region of autopsy material.[5] Other tumors in the sellar region are, in rank order, craniopharyngioma (3%), Rathke's cleft cysts (2%), metastases (<2%), Crooke's cell adenoma (<2%), and meningioma (1%) followed by an array of other lesions arising from structures in the sellar region (each comprising far less than 1%).[5]

1.2 PITUITARY NEUROENDOCRINE TUMORS

Tumors arising from the hormone-secreting epithelial cells in the adenohypophysis of the pituitary gland are members of the neuroendocrine tumor (NET) family, similar to hormone-producing tumors found in the gastroenteric tract, e.g. carcinoids, insulinomas, and gastrinomas. The five cell types that these tumors originate from are: gonadotroph, corticotroph, thyrotroph, somatotroph, and lactotroph cells.[6] According to the most recent update by the World Health Organization (WHO) 2017, these tumors are defined histologically by their expression of adenohypophyseal hormones (PRL, GH, ACTH, TSH, LH, and FSH) and specific transcription factors (steroidogenic factor-1 [SF-1], promoting gonadotroph differentiation; pituitary transcription factor-1 [Pit1], promoting somatotroph, lactotroph, and thyrotroph differentiation; and, lastly, T-box transcription factor TBX19 [T-Pit], promoting corticotroph cell lineages).[6] Sometimes, proliferative tumor markers are used, e.g. Ki67 and p53, for identifying tumors with more aggressive behavior. These proliferative markers are rarely elevated in PitNETs and their use in clinical practice is debated.[6,7]

1.2.1 CLINICAL PRESENTATION

Clinically, overt PitNETs manifest from a mass effect by expansive tumor growth with either excessive hormone secretion, i.e. functioning tumors, or without any hormone secretion, i.e. non-functioning (NF) tumors. Although NF-PitNETs are regarded as a single group in clinical practice, it is important to remember that they may originate from any of the cell types in the adenohypophysis, of which gonadotroph (80%) and corticotroph (15%) types constitute the vast majority.[8] Other cell lineages each comprise only a few percent of NF-PitNETs.[8] NF-PitNETs usually manifest clinically by symptoms related to a tumor mass effect on adjacent structures, e.g.

extrasellar extension superiorly may compress the nearby optic chiasm and thereby cause visual field defects.[9] Large tumors that extend even further superiorly may reach up to the foramina of Monro, which can cause hydrocephalus if obstructed. Since NF-PitNETs only manifest by symptoms related to a tumor mass effect, these tumors are often diagnosed at a late stage. Approximately 50% of these tumors have infiltrated the nearby cavernous sinuses upon detection, limiting the prospect of radical surgery.[10] It should be noted that symptoms related to a mass effect can be evoked by any sufficiently large PitNET regardless of any excess hormone secretions or not.

Functioning PitNETs manifest by excess hormone secretion that evoke characteristic syndromes in affected patients. Somatotroph tumors secrete excess amounts of GH, which through the hormone insulin-like growth factor 1 (IGF-1) present as acromegaly in adults and gigantism in pediatric patients. Thyrotroph tumors secrete TSH, which induces thyrotoxicosis that increases metabolic activity in affected patients, resulting in symptoms such as weight loss and increased heart rate.[11] Corticotroph tumors typically present as Cushing's disease or with symptoms suggestive of a hypercortisolism, which are defined by excess ACTH production. Lactotroph tumors, through excess PRL secretion, lead to oligo- or amenorrhea and galactorrhea in females, and with lethargy, decreased libido, and sexual dysfunction in males. Finally, gonadotroph tumors usually manifest as NF-PitNETs with symptoms related to a mass effect. On rare occasions, excess hormone production may lead to increased levels of testosterone in males and estradiol in females.

1.2.2 EPIDEMIOLOGY

Pituitary tumors, of which PitNETs are the most frequent, are among the most common neoplastic lesions in the central nervous system.[12] They represent around 10% of intracranial tumors[13] and around 25% of neurosurgical resections.[14] Autopsy material from individuals deceased from non-pituitary-related causes demonstrates a high prevalence of neoplastic lesions in the pituitary gland, ranging between 10-35%.[15-17] Importantly, these tumors are usually asymptomatic and small in size. In Buurman and Saeger's autopsy material of 334 pituitary tumors detected post mortem, the prevalence was 10.4% with a mean size of 1.5 mm.[17] Fewer than 1% were >10 mm.[17] Furthermore, tumor findings in this autopsy material were evenly distributed between genders.[17]

PitNET prevalence in the general population has also been estimated through studies based on radiological material. There is a notable variability in the results presented depending on the modality and specificity of the investigation protocol. For example, in patients assessed with standard magnetic resonance imaging (MRI) head examinations, lesions were found in 0.1-1.2% of scans.[18-20] In specific pituitary MRI examinations, the detection rate was approximately 10%.[21] In a 2004 meta-analysis, Ezzat and colleagues evaluated the prevalence of pituitary tumors in both imaging and autopsy studies, and found an overall prevalence of 16.7% (14.4% and 22.3% in autopsy and radiologic studies, respectively).[22]

Clinically, however, these tumors manifest far less often. Data for clinically reported PitNETs shows an overall prevalence of approximately 90 cases/100,000 inhabitants (range 75.5-155.6/100,000 inhabitants).[23-28] In contrast to the equal gender distribution of PitNETs presented in autopsy

material, clinically overt tumors are more frequently diagnosed in females (68% of cases, as a mean from 7 prevalence studies).[23-29] Lactotroph, corticotroph, and thyrotroph tumors all occur more frequently in females with a 4.9:1 ratio in lactotroph tumors, 2.8:1 ratio in corticotroph tumors, and 1.7:1 ratio in thyrotroph tumors.[11,30] For overall prevalence, lactotroph tumors were most frequently diagnosed (53%) followed by NF-PitNETs (31%), somatotroph tumors (12%), corticotroph tumors (4%), and thyrotroph tumors or unclassified tumors (1-2%).[11,23-29,31]

The incidence of PitNETs range between 3.9-7.4 cases/100,000 inhabitants /year.[25,27,29,30,32] Most of these studies have used the standardized incidence rate (SIR) based on the WHO standard population, a methodology used for facilitating comparison between populations with demographic differences. By comparing older to more recent epidemiological material, the incidence has increased in recent decades. Longitudinal studies from single geographic locations have also demonstrated an increased incidence in more recent study periods.[23,33] Before the introduction of high-resolution cross-sectional imaging (late 1980s), the incidence was reported around 1 case/100,000 inhabitants/year[33] compared to more modern material where the reported incidence was around 4 cases/100,000 inhabitants/year.[23,30,32]

In Sweden, PitNETs referred for tertiary care are reported in a national quality registry, Swedish Pituitary Registry (SPR).[34] Tumors matching the International Classification of Diseases (ICD)-10 code benign neoplasm in the pituitary gland (D35.2), acromegaly (E22.0), Cushing's disease (E24.0), or thyrotoxicosis (E05.8) are reported to the registry by clinicians confirming the diagnosis. Since private-financed healthcare is non-existent for managing PitNETs, the coverage rate is high. Data is presented on both regional and national levels to evaluate possible epidemiological differences. The data

released so far from the registry has indicated some differences in incidence between urban and more rural populations, with the highest prevalence reported from the more densely populated areas of Stockholm and Västra Götaland region (VGR).[34] This may reflect differences in access to healthcare providers, but may also be attributed to difference in diagnostic practices and possibly ambition in data reporting.[34]

1.3 STRUCTURAL IMAGING

In 1899, only 4 years after Wilhelm Roentgen described X-rays, Hermann Oppenheim demonstrated an enlarged sella turcica, indicative of a tumor, on plain film radiography in a patient with acromegaly. The first publication describing pituitary abnormalities was published in 1912 by A. Schuller.[35] However, expansive lesions in the pituitary could only be assessed by indirect tumor findings on plain film radiographs, e.g. calcifications and/or enlargement of the sella. It was not until the introduction of cross-sectional imaging such as computed tomography (CT) and MRI in the 1980s that pituitary tumors could be first assessed directly. MRI techniques for diagnosing pituitary lesions have witnessed a rapid evolution since they first came into use and have become the "gold standard" of pituitary imaging. The high image resolution and the good soft tissue representation make it a desirable tool for assessing tumors in the sellar region and for delineating tumor findings in adjacent structures.[36,37] Bone and calcified structures are better characterized by CT but is seldom used in routine clinical investigation; CT is therefore mostly used when an MRI scan is not possible, e.g. for claustrophobic patients or those with magnetic implants.

Today, standard protocols for assessing pituitary abnormalities usually include sagittal and coronal T1- and T2-weighted (W) standard Turbo Spin Echo sequences as well as dynamic and delayed contrast enhanced T1W sequences.[37] T1W sequences provide good anatomical representation and T2W sequences are useful for identifying fluid components such as blood products and cysts. Contrast agents are usually gadolinium-based macromolecules. The dynamic contrast series are usually proceeded in the coronal projection with multiple time points (0, 30, 60, 90, 120, and 180 seconds). Delayed contrast T1W sequences provide coronal and sagittal projections and are used for

assessing the cavernous sinuses and to characterize other sellar tumors. The normal anterior pituitary gland is isointense to gray matter on non-contrast T1W and T2W sequences. The posterior pituitary, the neurohypophysis, has an intrinsic high T1W signal but is hypointense on T2W.

The size of the normal pituitary gland changes depending on hormonal status and age. Generally, the pituitary gland reaches maximum size in young adults and then continuously decreases in size as the individual grows older. Hormonally active individuals, i.e. during puberty or pregnancy, demonstrate the largest glands, completely filling the pituitary fossa with a bulging, convex upper border. The maximum height of the gland is 8 mm in adult males, 9 mm in women, and 12 mm during pregnancy.[38] For tumor evaluation, radiologic assessment differs with respect to the size of the lesion, i.e. macroadenomas (≥10 mm) and microadenomas (<10 mm).[39]

1.3.1 MACROADENOMAS

It is characteristic for macroadenomas to be predominantly located within the sella with a bulbous suprasellar extension forming an hourglass shape sometimes referred to as the "snowman sign", created by the sellar diaphragm's belt like function. As the tumor grows, the normal pituitary becomes flattened and displaced superiorly or laterally to one side, but never inferiorly.[36,37] Pituitary macroadenomas can often be reliably visualized with standard MRI sequences and, thus, the task is rather to provide information vital for differential diagnosis and surgical planning. This includes a precise description of the location, extent, and structure of the tumor as well as vascularization and relation to surrounding anatomic structures. Confirmed invasion of adjacent structures increases the risk of intraoperative injury and limits the prospect of

complete surgical resection. However, this can, in many cases, only be confirmed by surgical exploration as MRI has poor ability to discriminate compression from real invasion.[40] Best radiologic evidence of invasive tumor growth is revealed by findings of internal carotid artery encasement in the cavernous sinuses, but an encasement of >67% is still needed to diagnose invasion with certainty.[41] Other indicators of invasion are expansion of the sella, inferior tumor expansion into the sphenoid sinus, or perforation of the sellar floor.

1.3.2 MICROADENOMAS

Tumors with a diameter <10 mm are defined as microadenomas.[39] Even though MRI provides high image resolution for soft-tissue lesions, the detection rate for smaller tumors is still low. Microadenomas <5 mm are often missed.[42] Most microadenomas are distinct, often rounded, hypointense regions in the anterior gland. However, many microadenomas are isointense to the unaffected pituitary gland on T1W images and can easily escape detection.[36] If no adenoma is visible on non-enhanced scans, the use of paramagnetic contrast medium can be helpful. Microadenomas are usually identified as areas of relative hypo-enhancement in scans after 1-2 minutes.[43] However, the resolution of dynamic images is relatively low and this technique increases the risk of image artifacts. Thus, many smaller microadenomas remain undetected on images before and after contrast enhancement; this can be a problem, particularly when evaluating patients with Cushing's disease and a suspected ACTH-secreting microadenoma. Approximately 40% of these tumors may avoid detection.[44-47]

1.3.3 POST-OPERATIVE MRI ASSESSMENT

Post-operative imaging should be performed at earliest 3 months after surgery.[36,37] The post-operative tumor cavity can be filled with blood up to days and even weeks after surgery. Also, packing material implanted to obtain hemostasis and contain hemorrhage takes several weeks to resorb.[48] Thus, an early post-operative scan might still show a considerable space-occupying lesion, which cannot be distinguished with certainty from residual tumor tissue. With a successful total tumor resection, the space previously occupied by tumor is with time successively replaced with cerebrospinal fluid (CSF). However, in the case of residual tumor tissue, post-operative imaging assessment has many challenges. The T1W signal intensity of the residual tumor should be the same as pre-operatively, but this does not reliably distinguish from other post-operative changes.[36] Because standard MRI sequences only provide morphological information, distinguishing these entities may only be assessed longitudinally by repeated scans to confirm tumor growth or resorption of packing material. Moreover, a variety of other post-operative artifacts and structural changes may further challenge post-operative image assessment. Drilling of the skull base can leave metal debris, which generates severe image artifacts. Autologous implants (such as fascia lata and fat) or heterologous packing materials may further complicate post-operative assessment.[37]

1.4 FUNCTIONAL IMAGING

General principles

In contrast to structural imaging, the fundamental principle in functional imaging is based on registering electromagnetic radiation emitted from a source within the body. This electromagnetic radiation is emitted from radioactive material, radiopharmaceuticals, that participate in a biological function within the body and thereby produce a map of that function, e.g. glucose metabolism, receptor density, protein metabolism, mineralization, rather than presenting an anatomical image of purely morphological information. Traditionally, radioactive gamma decay was registered in planar detectors, commonly known as scintigraphy or gamma cameras. This has been, by far, the most utilized method in functional imaging and is still widely used in today's clinical practice. During the 1970s, however, the field of functional imaging advanced rapidly through the introduction of the positron emission tomography (PET). This technique is based on radioactive beta plus (β^+) decay, where a proton converts into a neutron by emitting a positron. The positron creates a complex with a nearby electron, resulting in total annihilation that converts the two particles into high-energy gamma radiation. These resultant gamma rays carry a specific energy at 511 keV and are emitted at a 180° angle to each other, that are in turn registered by a detector that maps the decay to generate an image (Figure 3). This method enables high image resolution, three-dimensional imaging of the radioactive decay, which preferably can be fused with a conventional radiologic cross-sectional image (CT or MRI) for a high-resolution diagnostic hybrid image. Compared to the scintigraphic

modality, the decay can be quantified which enables more reliable comparison between scans.

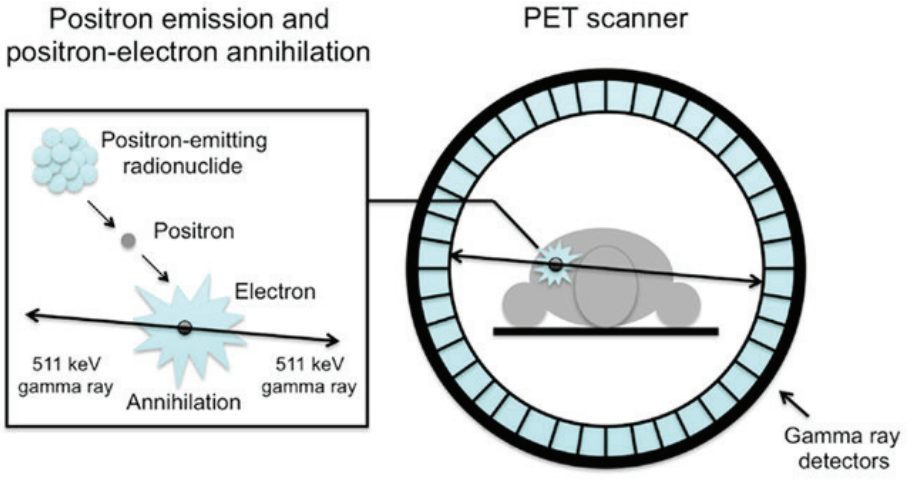

Figure 3. *Principle of a positron emission tomography (PET) camera. Positron-electron annihilation produces directly opposed gamma rays that are registered by detectors in the scanner. The site of annihilation is then calculated and mapped to create a three-dimensional image of the decay.*
Courtesy of van der Velt and collegues https://doi.org/10.3389/fonc.2013.00208. Licensed by CC BY 3.0

1.4.1 FUNCTIONAL IMAGING OF PITNETS

In the field of functional imaging, several approaches have been evaluated for the detection and management of PitNETs. The most commonly used PET tracer in the clinical setting for tumor detection is the glucose analogue ^{18}F-fluorodeoxyglucose (FDG) tracer, which visualizes glucose metabolism that is characteristically elevated in neoplastic lesions. Normally, the pituitary gland does not accumulate FDG and is therefore not visualized on FDG-PET imaging. Incidental uptake in the pituitary is also very uncommon. In larger cohort studies of patients who underwent whole-body FDG-PET scans,

incidental uptake was rare with a mean prevalence of only 0.2% (0.10-0.8%).[49-51] PitNETs were the most common cause of incidental uptake but only accounted about 50%.[49-51] Also, the rate of false positive uptake in these studies was as high as 30%. More focused studies that have evaluated FDG-PET found a sensitivity of 60-80%[52-54] in cases with confirmed PitNETs. The detection rate was as high as 100% uptake in macroadenomas but was generally low for microadenomas, around 50% of tumors.[52,53] FDG-PET does not seem to reveal any difference in uptake regarding different PitNET subtypes.[53] Even though FDG has advantages by being commonly available and providing a high sensitivity for larger tumors, the lack of specificity limits the tracer's potential use for PitNET detection.

Cellular receptors are also an interesting target for functional imaging. Promising results have been presented in detecting and monitoring medical treatment response in lactotroph and somatotroph tumors when targeting dopamine D2 receptors with ^{11}C-raclopride and 3-N-^{11}C-methylspiperone.[55,56] However, these receptors are mainly expressed by lactotroph tumors, which rarely need surgical treatment. Also, medical response can be reliably monitored by biochemical analyses rendering limited clinical use for this tracer. Regardless, these results are still interesting as a proof-of-concept for receptor imaging.

Other functional approaches to detect PitNETs include visualization of altered protein metabolism by radiolabeled amino acids. Most promising results have been yielded by the tracer ^{11}C-methionine (MET-PET), which is the most studied tracer for the visualization of amino acid metabolism so far. Unlike FDG-PET, MET-PET has the advantage of low uptake in adjacent structures to the pituitary gland, which yields a potential higher tumor-to-background ratio than FDG. MET-PET has reported high sensitivity for PitNETs and

provides important diagnostic information in situations where MRI, or even FDG-PET, fail to detect tumors, e.g. microadenomas.[57,58] Tang and colleagues assessed a group of 33 patients with biochemical or radiological evidence of pituitary adenoma recurrence after surgery.[59] In 14 of these patients, MET-PET detected residual tumor which was not visible on MRI. Koulouri and colleagues further demonstrated that co-registered MET-PET and MRI images facilitated targeted therapies of residual or recurrent somatotroph tumors and increased the rate of clinical and biochemical remission.[60] Rodriguez-Barcelo and colleagues studied 17 patients with newly diagnosed or surgically treated acromegaly using co-registered PET-CT and MRI images, reporting 86% sensitivity and 86% specificity for detecting recurrence after surgery.[61] Feng and colleagues compared FDG-PET and MET-PET in 43 patients with functioning PitNETs, finding a higher sensitivity with MET-PET.[57] Although this tracer demonstrated promising results with high image resolution and good tumor detection rate, the clinical use has remained very limited. The radioisotope ^{11}C, on which MET-PET is built, has a half-life of only 20 minutes, which limits its use to facilities with in-house cyclotron production. Thus, MET-PET has mainly remained a valuable tool for research purposes.

1.4.2 SSTR IMAGING

^{68}Ga-labeled tracers, on the other hand, can be generator produced, thus not requiring a cyclotron for isotope production (as needed for FDG, MET, and ^{11}C-D2 tracers), allowing for a broader use clinically. ^{68}Ga-labeled SST analogues, targeting SSTRs, have rapidly emerged as an established technique for detection and treatment monitoring in tumors that express SSTRs, e.g. NETs and carcinoid tumors.

SSTR imaging was first performed in humans in the late 1980s using ^{123}I-labeled octreotide. ^{111}In soon replaced ^{123}I. In the 1990s, a series of scintigraphic studies were conducted to evaluate the diagnostic properties of this technique in searching for PitNETs. The detection rate for positive scans varied from 60-71%,[62-65] with the best reported detection rate in somatotroph macroadenomas.[65] Although there were promising results in macroadenomas, the technique was poor for detecting microadenomas because of poor image resolution and a low tumor-to-background ratio. In addition, long scanning times, which was determined optimal after 2-24 hours,[64,65] also explains why this scintigraphic technique never found widespread clinical use.

Contrary to scintigraphy, the PET technique provides short scanning times and a low effective dose to the patient. It is also superior to scintigraphy in that it provides three-dimensional imaging with high spatial resolution. In addition, it offers the ability to quantify tracer uptake using a semi-quantitative measure of standardized uptake values (SUVs), which enables the monitoring of medical treatment response and facilitates reliable comparison with earlier imaging results.

In NETs, which like PitNETs also express SSTRs, ^{68}Ga-labeled SSTR-PET has become a standard imaging modality.[66] In a number of studies, the technique has demonstrated far superior sensitivity and specificity over scintigraphic technique modalities, while also providing shorter scanning times and a lower effective dose.[67-71] The SSTR expression in PitNETs have been described to vary regarding PitNET subtype. For example, thyrotroph and somatotroph tumors express SSTR2 and SSTR5 to a high degree,[2,72] whereas corticotroph and gonadotroph tumors exhibit a higher degree of SSTR3 expression and a more variable degree of SSTR5 expression.[73] Although

PitNETs express these receptors and SSTR-PET has emerged as an established technique for related tumors, this technique has not been sufficiently evaluated.

Recently, smaller pilot and case studies evaluating ^{68}Ga-labeled SSTR-PET in PitNET detection have shown promising results. The utility of ^{68}Ga-DOTANOC PET/CT for revealing ectopic PitNETs has been shown in two separate case reports.[74,75] In a systematic review of patients with ectopic Cushing's syndrome, Isidori and colleagues described SSTR-PET as having the highest sensitivity (81.8%) among functional imaging modalities. Also in the same study, the functional imaging modalities outperformed conventional radiologic modalities, in finding 79.1% of tumors missed by conventional radiology.[76] SSTR-PET in combination with FDG-PET has also demonstrated high sensitivity and specificity in corticotroph microadenomas in the pituitary gland.[45] This multi-tracer approach was also helpful in the post-operative assessment of patients with surgical treatment: SSTR-PET was able to correctly identify a pituitary tumor in nine of 12 patients with multiple endocrine neoplasia type 1.[77]

However promising the results in smaller studies, the field still lacks systematic studies evaluating the utility of SSTR-PET in PitNETs generally and in its specific subtypes. The high physiological uptake of SST analogues in normal pituitary glands is of concern when evaluating pituitary neoplasms, especially microadenomas, and must be addressed. The high resolution of modern PET cameras may allow the technique to overcome these obstacles.

1.5 KNOWLEDGE GAPS

Epidemiology

PitNET epidemiology has not been studied in VGR since the 1980s. Since then, diagnostic procedures have witnessed rapid development, in particular with high resolution imaging and ultra-sensitive biochemical analyses. Contemporary PitNET incidence from other countries indicate a surge in the number of cases, particularly for smaller tumors found incidentally. Nonetheless, this trend needs to be validated in a large population with excellent record-keeping and up-to-date registries.

Existing data from the SPR suggest an under-reporting of lactotroph tumors in females, which might correspond to a loss of female patients with mild hyperprolactinemia being treated at local and/or privately organized gynecologists. Also, the discrimination of NF-PitNETs with mild hyperprolactinemia from lactotroph tumors is challenging and contributes to discrepancies in data reported from different materials. Lastly, epidemiological data from the SPR relies on active reporting from the clinicians making diagnoses. There is sometimes flaws in the quality of this data, since reporting might be incorrect or data can be missed when working in limited timeframes. Complementing the SPR with data from thorough scrutiny of clinical material is most important for providing up-to-date data for clinicians and healthcare organizers.

Structural imaging

Standard structural imaging with MRI and/or CT provides sufficient information for the clinician for managing the majority of PitNET cases. However, routine clinical sequences do not always reliably identify the site of a *de novo*, persistent, or recurrent PitNET, which in turn may reduce the

prospect for curative surgery. Moreover, the lack of functional information in conventional structural imaging modalities, demands repeated, longitudinal imaging assessments to determine possible tumor growth. Also, tumor growth is often determined as an increase in size of ≥2 mm,[78] which is not easy to conclude if the imaging sequences do not match perfectly or are acquired in a different angulation.

Functional imaging

Although there are a multitude of promising results in PET pilot studies, the technique has yet to become widely accepted in clinical practice. Most tracers are expensive and their availability is limited. More commonly used and available tracers, like FDG, are limited by low specificity. On the contrary, ^{68}Ga-labeled SST analogues are both relatively more available and have demonstrated promising tumor specificity for SST-expressing tumors. However, the tracer still needs to be a systematically assessed before possible introduction into clinical routine. Also, since the expression of SSTR is a defining characteristic of PitNETs, many of these tumors are medically treated with SST analogues with varying results. An imaging modality that could predict possible medical response would be of great benefit for the clinician and, ultimately, patients.

2 AIM

Paper I

- To study the SIR of clinical presenting PitNETs in western Sweden from 2001 to 2011.

Paper II-III

- To study the diagnostic properties of ^{68}Ga-DOTATOC PET in PitNETs that require surgical treatment, i.e. somatotroph, thyrotroph, corticotroph, and gonadotroph tumors.
- To study the ability of ^{68}Ga-DOTATOC PET to discriminate between residual tumor tissue from scar tissue and normal pituitary after surgery.

3 PATIENTS AND METHODS

3.1 PAPER I

Study design

This was a retrospective observational study including all PitNETs diagnosed within endocrine departments and reported to the pituitary registry in VGR from 2001 to 2011.

Registry and target population

The SPR is a national quality registry, established in 1991, where information on basic demography, clinical findings, biochemical analyses, radiology, histopathology, treatment, and outcome in patients with PitNETs are collected. VGR is located in the southwest of Sweden and has a well-defined population of approximately 1.7 million inhabitants (Statistics, Sweden) with one university hospital and four regional hospitals with endocrine departments.

Patients

Patients living in VGR diagnosed with PitNETs between 2001 and 2011 were included in this study. The population register for VGR was used to confirm that the patients were resident in the region. In addition to existing data from the SPR, a search in the diagnosis-related group (DRG) registries at the Departments of Medicine in Alingsås, Borås, Skövde, Uddevalla, Trollhättan, and Göteborg was conducted with the ICD-10 codes for benign neoplasm of the pituitary gland (D35.2), acromegaly and pituitary gigantism (E22.0), hyperprolactinemia (E22.1), Cushing's disease (E24.0), and thyrotoxicosis (E05.8). If a tumor was confirmed in medical records, patients were then included in the SPR if not previously registered. After completed registration,

the information on patients with PitNETs fulfilling the inclusion criteria was collected from the SPR. Besides inclusion in the SPR, patients were eligible for inclusion in the study if the diagnosis was established after the age of 18 years, the patients were living in VGR at the time of diagnosis, and the diagnosis was established between 2001 and 2011.

Methodological considerations

The SPR coverage of patients with PitNETs is considered high. There is no private healthcare sector for managing these tumors and most newly discovered PitNETs are referred to secondary and tertiary care providers for a reliable diagnosis. However, there are still factors that confound the coverage of PitNET patients that need to be considered. First, patients with mild hyperprolactinemia are suspected as being missed from the registry, as these patients are sometimes treated medically by gynecologists. Also, smaller lesions in the pituitary gland found incidentally with MRI or CT may evade reporting to the registry. Normally, the coverage is higher for symptomatic tumors that reach tertiary care, where reporting to cancer registries is more routinely executed.

To increase the coverage in the registry, we added missing cases after a thorough review of clinical records for patients who were found searching DRG registries.

3.2 PAPERS II-III

Study design

Pre-operative part

Case-control, prospective, pilot study of patients with PitNETs eligible for surgical treatment who were evaluated with ^{68}Ga-DOTATOC-PET compared to healthy control subjects before surgical treatment.

Post-operative part

Longitudinal comparison of pre- and post-operative findings of ^{68}Ga-DOTATOC-PET in patients with PitNETs. Also, ^{68}Ga-DOTATOC uptake was analyzed with respect to histopathological classification and correlated to individual SSTR expression. For TSH and GH PitNETs, a 3-year post-operative follow-up PET and MRI scan will also be conducted.

Subjects

In total, 31 adult patients with a treatment-naive (including surgery, SST analogues, or dopamine agonists) PitNET and 16 healthy control subjects were included in this study. Patients were included in the study at clinical presentation: ten patients had NF-PitNETs, nine acromegaly, eight Cushing's syndrome, and four hyperthyroidism and suspected TSH-producing PitNET.

The patients were recruited from university hospitals in Sweden after being diagnosed and elected for surgical pituitary treatment. The diagnoses were based on MRI evidence of a pituitary tumor, biochemical analyses, and clinical presentation that met the criteria for ICD-10 code benign neoplasm in the pituitary gland (D35.2) and/or acromegaly (E22.0), Cushing disease (E24.0), or thyrotoxicosis (E05.8). Patients with lactotroph tumors were not included, as they rarely undergo pituitary surgery.

Two groups of control subjects (*n*=16) were selected. The first group included 13 healthy volunteers who were randomly selected from the population registry in Gothenburg, Sweden. To be included in the evaluation as a control, the MRI had to be negative for incidentalomas in the pituitary. The second group included three controls with thyroid-associated ophthalmopathy who were participating in another study where ^{68}Ga-DOTATOC-PET was performed using the same scanning protocol as in our patients to evaluate eye muscle inflammation. The MRI was performed according to the clinical assessment protocol at the Department of Radiology, Sahlgrenska University Hospital, Gothenburg, Sweden, including but not restricted to a T1W scan with and without gadolinium contrast enhancement as well as a non-contrast-enhanced T2W sequence. Additionally, a three-dimensional contrast-enhanced T1W sequence was acquired for optimal co-registration with the PET images. Post-operative MRI scans were assessed together by an experienced neuroradiologist with respect to residual tumor tissue and post-operative changes. Post-operative MRI was conducted at 6-8 months after pituitary surgery.

^{68}Ga-DOTATOC-PET

^{68}Ga-DOTATOC-PET were performed at the Department of Nuclear Medicine at Sahlgrenska University Hospital using a CT Flow Edge PET/CT scanner (Siemens Medical Solutions USA, Inc.). CT and PET scanning parameters are stated in detail in papers II and III. The PET scan included a dynamic list-mode acquisition starting at the time of tracer injection to collect emission data over a 45-minute period and, finally, a static 300-second image was acquired 60 minutes post-tracer injection. The post-surgical PET scan was performed with the same study protocol at least 6 months after pituitary surgery to avoid any interference in uptake due to inflammation.

Image analysis

PET and MRI images were co-registered using the "Fuse it" toolkit in PMOD software v3.8 (PMOD Technologies, Ltd., Zürich, Switzerland). Tumor findings were outlined in transverse slices, creating a volume of interest (VOI) around the tumor area. For the control group, the VOIs were outlined around the pituitary gland. The VOIs in the post-surgical scans were created in the same manner as in the pre-surgical analysis. Tracer uptake was analyzed in the VOI with regard to maximum SUV (SUV_{max}). In the post-operative imaging assessment, no measurements were performed in patients without any abnormal MRI findings, who were considered cured by a combination of biochemical and clinical factors.

Histopathology

Resected tumor tissue was collected during surgery and representative tumor tissue was confirmed in routine hematoxylin/eosin-stained sections from formalin-fixed, paraffin-embedded tissue blocks. After finishing the routine diagnostic process, stained and unstained sections were sent to a specialized pituitary pathologist at Uppsala University Hospital to evaluate morphology, hormone expression, Ki67 index, and SSTR expression using specific monoclonal antibodies. The PitNETs were classified into histological subtypes according to the 2017 WHO classification based on the immunohistochemical expression of anterior pituitary hormones and pituitary-specific transcription factors.[6] Plurihormonal tumors were categorized as to their clinical presentation. Immunoreactive score (IRS) was used for the quantification of SSTR expression in tumors.[73,79] This scoring system is defined as the product of the proportion of immunoreactive cells (0 = 0%, 1 = <10%, 2 = 10-50%, 3 = 51-80%, and 4 = >80%) and the staining intensity (0 = no staining, 1 = weak; 2 = moderate, and 3 = strong).

Methodological considerations

As a consequence of studying rare tumors while keeping a reasonable time-line, this study is limited by the relatively small sample size. Also, as PET, MRI, and histopathological assessments are relatively expensive modalities, the number of patients needed to stay reasonably limited. At first, we aimed to recruit all patients regionally, but the slow pace of inclusion led us to broaden recruitment of rare hormone-producing PitNETs to a national basis. The number of patients in our study is, however, similar to other PET-oriented pilot studies and also sufficient to determine significant differences in tracer uptake in tumors and the pituitary gland.

Regarding the imaging methodology, there are several limitations in our research protocol. First, we did not decide on a fixed MRI investigative protocol and there was no neuroradiological expertise engaged in developing the research protocol. Initially, most patients were thought to be recruited from the Sahlgrenska University Hospital alone, thereby resulting in a uniform investigation protocol. However, recruitment was later extended to other regions and, additionally, some regional MRI evaluations were outsourced to private concerns, consequently resulting in the use of different MRI protocols. To balance this confounding factor, supplementary MRI investigations were assigned if the quality was insufficient or necessary sequences were missing. This lack of uniformity is not optimal in a research situation but, most certainly, it did not affect the outcome as all MRI and PET images could be effectively co-registered and tumors clearly delineated.

The scanning time was originally performed up to two hours but was later reduced to one hour after the initial 12 scans. Demand for the PET camera grew during this research project and after the initial scans did not show indisputable evidence for a prolonged 2-hour scanning time, we could not decisively argue

to maintain this long scanning protocol. Also, some patients experienced some difficulties to endure the long scanning protocol, which became a factor to consider when recruiting patients.

On a more technical note, there are inherent limitations with the PET technique. A partial volume effect could be a confounding factor when measuring tracer uptake, especially in smaller lesions. Also, the positron range limits image resolution and precise measurements. ^{68}Ga has a particularly high positron energy (E_{mean}= 0.83 MeV), which yields a long positron range (R_{mean} 3.5 mm),[80] resulting in lower imaging resolution.

A major strength with our method is the reliable histopathological data that complements the uptake data. Other PET studies rarely present any histopathological data as a basis for the uptake profile. Also, the histopathological data in our study used modern monoclonal antibodies which provide much more reliable results without the risk of cross-binding, as was the risk with older polyclonal antibodies used before 2012.[73]

3.3 STATISTICAL METHODS

Paper I

The analyses of the incidence rates were based on the assumption of a Poisson distribution. The annualized incidence rate was age standardized to the WHO 2000 standard population[81] and is given as rates per 100,000 people with 95% confidence intervals (CIs). Differences in incidence rates between 2001-2005 and 2006-2011 were analyzed by Poisson regression, accounting for age and gender. Categorical variables are presented with numbers and percentages, and continuous variables with means and standard deviations. For comparison between groups, Fisher's exact test was used for non-ordered categorical variables and *t*-test for continuous variables. A two-sided $P < 0.05$ was considered statistically significant. For all analyses, the statistical software package Stata version 12.1 was used (StatCorp. 2012. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP).

Papers II-III

Due to small sample sizes and the nature of image data distribution, non-parametric statistics were used. Variables are presented as medians and interquartile ranges (IQRs). Mann-Whitney *U*-test was used to compare median SUV_{max} in patients and controls. Receiver Operating Characteristic (ROC) analysis was used for evaluation of the diagnostic accuracy. Since this was a pilot study, there is no previous data on which to base power calculation. Spearman's rank-order correlation was used for correlation analyses between SSTR expression and SUV_{max}. For all tests, $P < 0.05$ was considered as statistically significant.

3.4 ETHICS

Paper I

The study was approved by the Ethics Committee (675/03 and 2012/915-32) in Stockholm, Sweden. This study was performed in accordance with the Declaration of Helsinki.

Papers II-III

The Regional Ethical Review Board in Gothenburg, Sweden, approved the study (Dnr 393-10; 2010-08-06), which was performed in accordance with the Declaration of Helsinki. Approvals were also obtained from the Medical Product Agency and the Radiation Safety Committee. All patients and control subjects signed informed consent before participating in the study.

Ethical considerations

Paper I

The epidemiological data in this study were taken from the SPR. There are always concerns of integrity when including and presenting data from a medical registry. The data reported to SPR include the patient's personal identification number but all information is classified. The data is then encrypted and stored on internal servers at the regional internal network. Only the regional representative, who has access to the personal identification numbers can possibly identify patients. Data presented from this registry is always anonymized and presented at group level.

The value of keeping quality registers is an improved detection for systematic differences in diagnosis and treatment outcome with the end result in quality improvement. This is essential information for organizers of healthcare and for accurate distribution of medical resources. There is no direct benefit for the

patient at a personal level but the increased knowledge benefits all future patients with the diagnosis.

Papers II-III

All patients and control subjects receive a radioactive dose for every PET scan. This puts patients at risk for possible acute and/or late side effects. The effective dose received by each PET scan is approximately 10-12 mSV and does not exceed 18 mSV, which was the upper limit given by the ethical approval. This dose is not considered high but needs to be medically motivated to be considered ethically appropriate. The medical benefit of this research project should increase accuracy, which would shorten primary diagnostic work-up and post-operative evaluation. Also, it has the potential to limit the use of invasive diagnostic procedures such as sinus petrosus catheterization, which puts patients at a greater acute risk than a low dose of electromagnetic radiation. Since patients with PitNETs are usually monitored by an MRI scan, the total yearly effective dose received during routine clinical investigation is low and far below the upper safety limit of 50 mSV/year.[82] Also for the control subjects, the effective dose per year would stay within safe limits. In conclusion, we consider the possible medical benefits from this research project to far outweigh the risks associated with this method.

There are, of course, other ethical dilemmas in using an expansive diagnostic method and the increased number of clinical investigations for patients already committed to comprehensive clinical testing. Can we justify the costs? Can we justify the increased burden of investigations for these patients? First, the cost for each PET scan is relatively high compared to other modalities, as is the nature of a relative newly introduced technique. By comparison, both CT and MRI were very expensive modalities when first introduced but have since witnessed a rapid decline in cost over recent decades. As a technique is

increasingly utilized, the cost usually drops when the technique is being scaled up. ^{68}Ga-labeled PET tracers have the potential for broad clinical use as a generator-produced isotope and thus eliminates the need for a nearby cyclotron, which is analogous to the role ^{99m}Tc has in scintigraphic techniques.

By confirming the potential clinical use of ^{68}Ga-DOTATOC for PitNET detection, the number of clinical visits for patients as well as the investigation time could be greatly reduced, with a subsequent overall cost reduction of the clinical management of PitNET patients. Furthermore, potentially, the cost for other ^{68}Ga-labeled tracers could also be reduced on the basis of the rationale of scale.

4 RESULTS

4.1 PAPER I

Standardized incidence of PitNETs

In VGR from 2001-2011, 592 adult patients in the SPR fulfilled the inclusion criteria. The overall SIR of PitNETs during the study period was 3.9 cases/100,000 inhabitants/year (95% CI 3.6-4.3). There was no significant change in the SIR during the study period. The mean number (range) of patients diagnosed with PitNETs per year was 54 (42-69): 26 (17-36) in men and 28 (17-34) in women. During the 11-year study period, the time-trend did not demonstrate any significant change in the overall incidence rate.

Age- and gender-related incidence of pituitary adenomas

The distribution was slightly skewed towards females, who demonstrated a higher SIR (4.7/100,000/year; 95% CI 4.1-5.3) compared to men (3.3/100,000/year; 95% CI 2.9-3.7) and who also comprised 52.2% of all patients. Females were younger at the time of diagnosis compared to men (mean age 46.2 *vs* 57.8 years, respectively). In males, the frequency of PitNETs increased with age, which peaked in the oldest age group (>75 years), and was mainly attributed to NF-PitNETs. In women, the highest incidence of PitNETs was in the age range 25-34 years, mainly due to a high incidence of lactotroph tumors.

Incidence by PitNETs subtype

The incidences of PitNETs by subtype are shown in Figure 4.

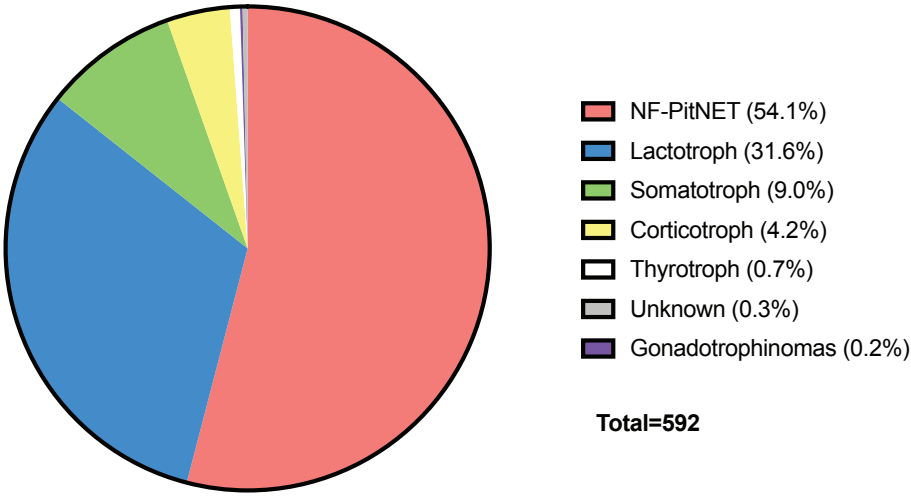

Figure 4. *Pie chart demonstrating the proportion of PitNETs subtypes diagnosed in VGR from 2001-2011.*

NF-PitNETs

NF-PitNETs were most commonly diagnosed, which accounted for 54% (*n*=320) of all cases, and was more frequent in men than in women. The incidence increased with age in both genders.

Lactotroph tumors

Lactotroph tumors comprised 32% (*n*=187) of cases. The SIR for lactotrophs was 1.6/100,000/year (95% CI 1.3-1.9) and was higher in women (2.4/100,000/year; 95% CI 1.9-2.9) compared to men (0.9/100,000/year; 95% CI 0.6-1.1). Lactotroph tumors were most frequently diagnosed in females at a fertile age. No relevant differences in incidence were seen between males and post-menopausal females.

Somatotroph tumors

As the third most common subtype of PitNET, somatotroph tumors accounted for 9% (*n*=53) of all tumors. The SIR for acromegaly was 0.35/100,000/year (95% CI 0.25-0.45) with no significant difference between genders.

Corticotroph tumors

Corticotroph tumors accounted for 4% (*n*=25) of all tumors and were more frequently diagnosed in women. The SIR was 0.18/100,000/year (95% CI 0.11-0.25): 0.29/100,000/year (95% CI 0.16-0.43) in females and 0.07/100,000/year (95% CI 0.01-0.13) in men. The incidence of Cushing's disease was highest in the age groups 25-44 years and 55-64 years.

Thyrotropinomas and active gonadotropinomas

TSH-producing PitNETs represented 0.7% (*n*=4) of all cases. The SIR for thyrotropinomas was 0.03/100,000/year (95% CI 0.00-0.05). Only one patient was diagnosed with a clinically overt gonadotrophinoma during the study period.

Macro- and microadenomas

Macroadenomas were more frequently seen (65%; *n*=385) than microadenomas (33%; *n*=198). Overall, macroadenomas were more frequently seen in males (82%) than among females (50%). Macroadenomas comprised the vast majority of NF-PitNETs, and somatotroph and thyrotroph tumors. Only 37% of all lactotroph tumors were macroadenomas despite the vast majority (68%) of lactotroph tumors in men being macroadenomas. In corticotroph tumors, 28% of the tumors presented as macroadenomas with no significant difference between males and females (33% *vs* 26%, respectively).

4.2 PAPERS II-III

Tracer uptake

Due to being unpublished data, detailed information is to be found in paper II-III. Tracer uptake in normal pituitary gland from the control subjects was homogenous and fairly uniform at approximately SUV_{max} 14. Highest tumor uptake was seen in thyrotroph tumors where uptake was significantly higher compared to normal pituitary gland. High levels were also seen in somatotroph tumors but uptake was only discriminated from normal pituitary with statistical significance after 1 hour. Corticotroph and gonadotroph tumors demonstrated significantly lower tumor uptake compared to the normal pituitary gland. Representative examples of PET/MRI scans are shown in Figures 5-8.

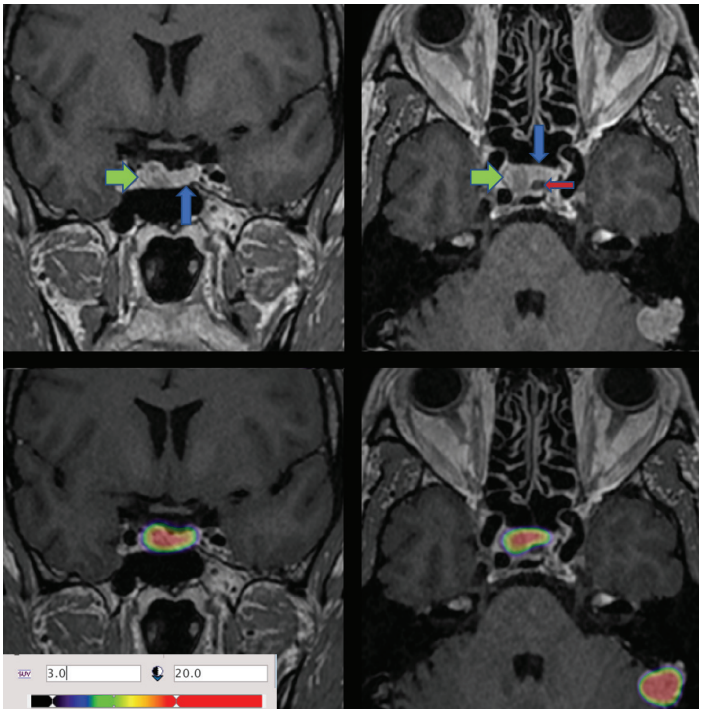

Figure 5. *Patient with a somatotroph tumor. MRI demonstrates a 5 × 10 mm hypo-enhancing focus (green arrow) in the right side of the pituitary gland. Normal pituitary tissue demonstrates a normal contrast-enhancing pattern (blue arrow). A 5-mm cyst is also seen in the posterior part (red arrow). Incidental tracer uptake is seen in the left sigmoid sinuses, corresponding to a verified meningioma.*

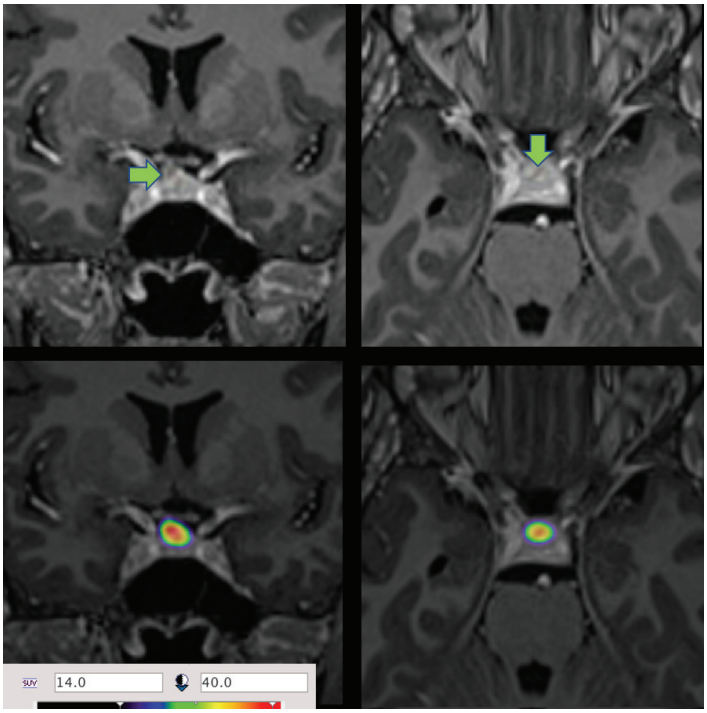

Figure 6. *Patient with a thyrotroph tumor. An approximately 5-mm rounded lesion (green arrow) is seen in the superior part of the pituitary gland.*

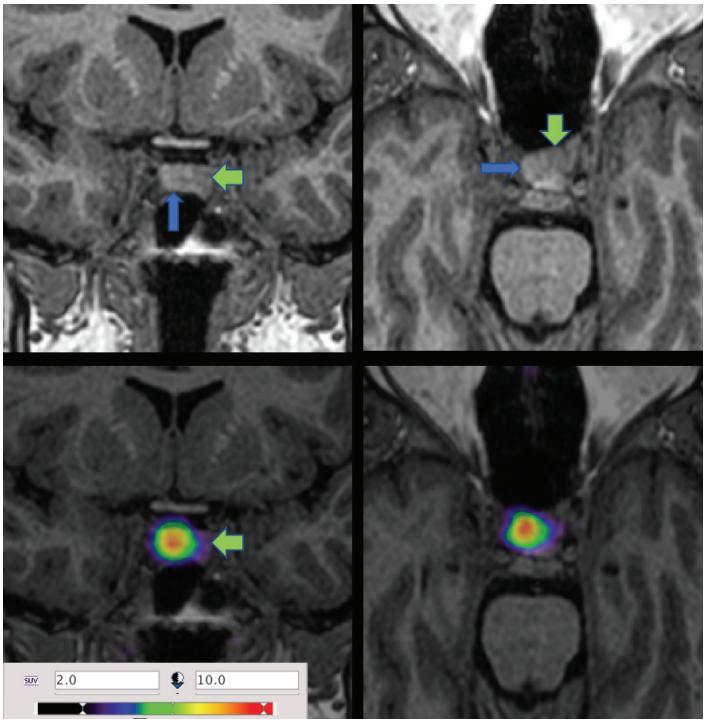

Figure 7. *Patient with a corticotroph tumor in the left side of the pituitary gland (green arrow). Normal pituitary tissue demonstrates a normal contrast-enhancing pattern (blue arrow). In addition, tracer uptake here is higher in normal pituitary tissue compared to tumor tissue (green arrow).*

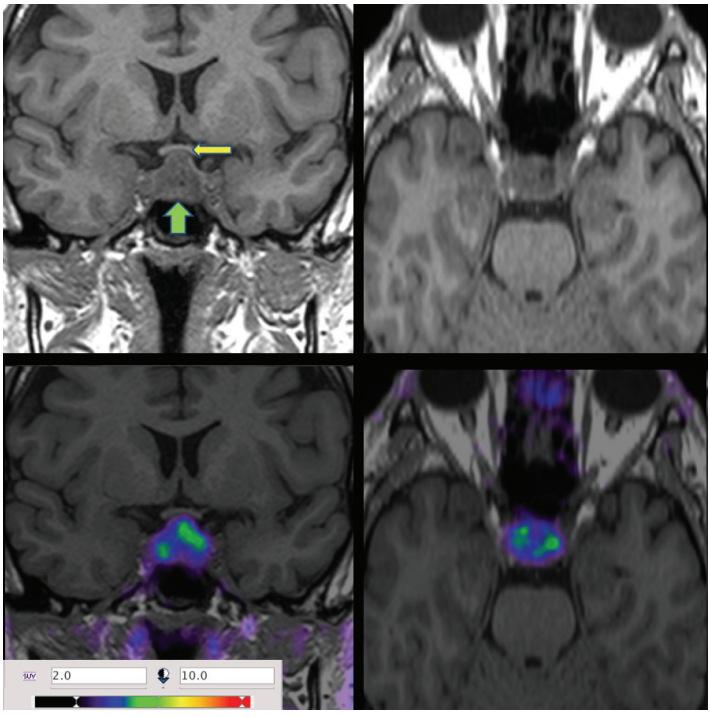

Figure 8. *Patient with a large gonadotroph tumor (green arrow) compressing the optic chiasm (yellow arrow).*

Tracer kinetic properties

In the normal pituitary gland, tracer uptake demonstrated a gradual rate of uptake that peaked at 45 minutes. Somatotroph tumors demonstrated an uptake pattern that was similar to normal pituitary gland but continued to increase after 1 hour. Corticotroph and gonadotroph tumors did not demonstrate any significant increase in uptake over time. In contrast, thyrotroph tumors showed a steadily increased uptake that continued to increase after 1 hour (Figure 9).

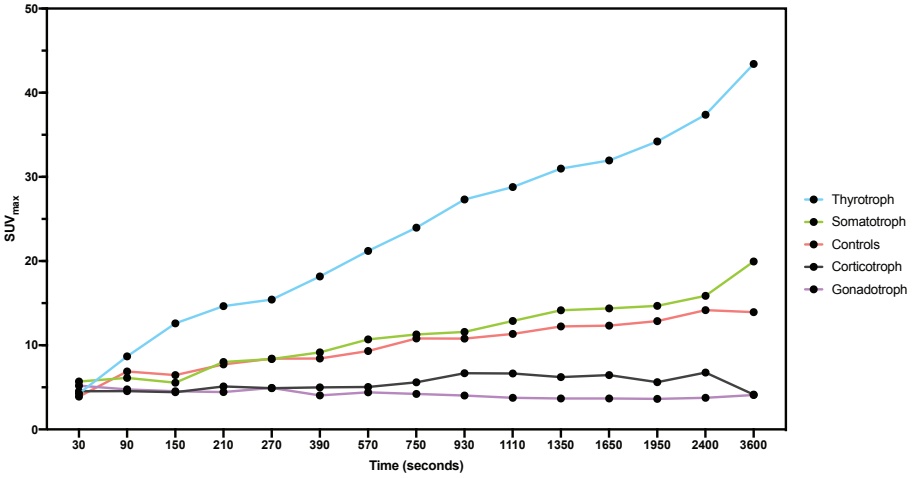

Figure 9. *Kinetic uptake profiles for normal pituitary gland (red line) and PitNET subtypes over the 1-hour time frame.*

Histopathological assessment

Full details are provided in Paper II and Manuscript III. Histopathological assessment demonstrated seven somatotroph, three thyrotroph, seven corticotroph (two clinically non-functioning), and seven gonadotroph (all clinically non-functioning) tumors. The expression of SSTR2 and SSTR5 was high in somatotroph and thyrotroph tumors. SSTR3 was the predominant receptor for corticotroph and gonadotroph tumors.

Correlation of tracer uptake and SSTR expression

A strong correlation was found between SSTR2 and tracer uptake levels (presented as SUV_{max}). A positive relationship was found between SSTR5 and tracer uptake but was not statistically significant. For SSTR1 and SSTR3, no correlation was found with tracer uptake.

Post-operative assessment

Eleven patients showed an abnormal post-operative MRI with findings of either a suspected residual tumor tissue or suspected scar tissue. In most cases, the modality could not reliably distinguish between residual tumor tissue and scar tissue. However, the tracer was found to be useful when comparing post-operative tumor uptake with pre-operative uptake in the same patient. To accurately separate tumors from scar tissue, tumors with higher uptake than in the normal pituitary were chosen, at a threshold uptake level of >13.8 SUV_{max} (corresponding to the median uptake in normal pituitary gland). In these tumors (*n*=4), an uptake <60% of pre-operative uptake corresponded with clinical remission in all four cases. Tumors with a persistent uptake >60% of pre-operative uptake corresponded with unaffected hypersecretion in both cases (Figure 10). This interesting finding needs to be further evaluated in a larger cohort.

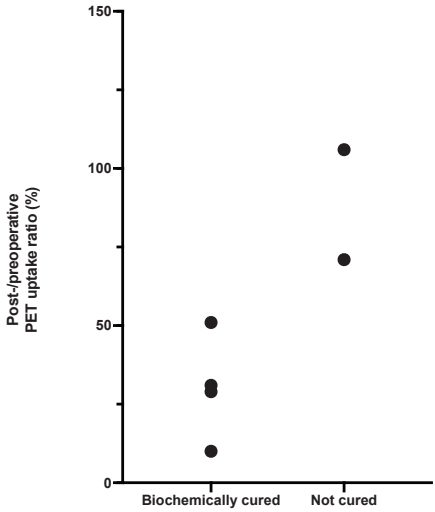

Figure 10. *Post-operative uptake profiles for six patients with a pre-operative tumor uptake of SUV_{max} >13.8. Four patients with a post-operative uptake with <60% of pre-operative uptake corresponded with biochemical cure. Conversely, in two patients with tumor uptake >60% of the pre-operative uptake, the uptake corresponded with a persistent tumor.*

5 DISCUSSION

5.1 PAPER I

With a target population of 1.6 million, at the time of the study, and a cohort of 592 patients with PitNET, this is to date the world's largest study investigating the incidence of PitNETs. The primary aim was to determine the SIR for PitNETs over 2001-2011, which was 3.9 cases/100,000 inhabitants/year. This finding is much higher than that previously reported from the same region over 1958-1991, when cross-sectional imaging was not commonly available and diagnosis was based on less sensitive biochemical methods.[33] Other studies evaluating PitNET incidence in modern clinical settings have recently presented similar results with increased incidence rates.[23,25,27,29] There are, however, some differences in the incidences reported in these studies with SIRs ranging between 3.9-5.8/100,000/year. The highest incidence was reported from a nationwide Icelandic study, which found an almost 50% higher SIR than other studies including ours.[25,30] Interestingly, symptomatic hormone-producing tumors seem to be fairly consistent in these studies but the number of NF-PitNETs varies greatly. Since most NF-PitNETs are small and asymptomatic, studies that reported a higher overall SIR also reported a higher proportion of incidentally detected NF-PitNETs, which presumably could be related to a more frequent use of high-resolution imaging. In summary, this difference in incidence in modern studies suggests methodological differences in detecting and reporting cases rather than a true difference in the incidence related to environmental or genetic factors.

In our study, it is difficult to conclude an increased incidence rate of PitNETs for the study period. This would be expected from the relatively short study period of only 11 years, during which there was consistent use of diagnostic

instruments over the whole study period. However, compared to Nilsson and colleagues,[33] who studied the same target population from an earlier period, there are some interesting differences. Most notable is the increased incidence seen in younger women. In Nilsson's study, the age-specific incidence peaked between 60-70 years in both men and women[33] compared to a peak incidence at 25-35 years for females in our study.[32] This is most likely attributable to an increased incidence in PRL-producing tumors; unfortunately, no data regarding tumor subtypes was presented in Nilsson's study. The incidence also increased for men but the age-specific peak incidence remained in the highest age group (>70 years).

NF-PitNETs

We found NF-PitNETs to be the most common subtype with an overall incidence of 1.8/100,000/year, comprising 54.1% of all tumors reported. This is higher than most epidemiologic studies in the modern era. NF-PitNETs comprise around 30% of all PitNETs, as reported from seven epidemiologic studies from 2006-2016.[23-29] In relation to an epidemiological study from northern Finland, a region similar to VGR with respect to healthcare organization and population, there is a prominent difference in the proportion of NF-PitNETs. Notably, the combined proportion of both lactotroph tumors and NF-PitNETs adds up to roughly the same number in both studies: 85.7% in our study[32] compared to 88% in the study by Raappana and colleagues.[30] This points, somewhat, to methodological differences when classifying these tumors. An elevated PRL level is known to occur in a phenomenon called pituitary stalk compression. Expansive processes in the pituitary gland often compress the pituitary stalk, thus interrupting the delivery of dopamine that exerts an inhibitory effect on PRL secretion. Since most prolactinomas are medically treated, a verified histopathological diagnosis is rarely seen. Elevated serum PRL levels could therefore be attributed to both true lactotroph

tumors and NF-PitNETs with pituitary stalk compression. The cut-off level for distinguishing these tumors has long been a topic of debate but a recent, well-designed study suggested a 3.5-times upper reference PRL limit >1000 mIU/L (47.2 ng/mL) in males and >2000 mIU/L (94.3 ng/mL) in females.[83] In our study, a PRL-producing lactotroph tumor was defined when PRL levels were >3 times the upper reference limit. Consequently, tumors >3 mm and with concurrent hyperprolactinemia were classified as NF-PitNETs if hormone levels were below this >3 times threshold level. We therefore claim to present more accurate incidence data regarding true lactotroph tumors compared to other epidemiological studies with no regard to discriminatory prolactin threshold levels.

Lactotroph tumors

Lactotroph tumors accounted for 31.6% of tumors with an overall incidence of 1.6/100,000/year. As stated above, the proportion of prolactinomas is lower as a consequence of the high PRL threshold level used in our study. Additionally, there is a known limitation in our study of under-reporting of lactotroph tumors. This is mainly explained by the fact that patients with (mild) hyperprolactinemia are often medically treated by local gynecologists, of whom many are in the private sector. The true incidence of prolactinomas is assumed to be higher, especially when lactotroph tumors are among the most common PitNET subtype found in autopsy material.[15,17] Lactotroph tumors were more frequently diagnosed in females with a peak incidence in the 25-34 year age group. Interestingly, the incidence of lactotroph tumors is roughly the same in males and females after menopause, as is the distribution of lactotroph tumors in autopsy material.[84] The difference in incidence seen over fertile ages may, to some extent, be explained by the different clinical manifestation in genders. In females, the main symptoms are amenorrhea, galactorrhea, and infertility, which perhaps motivate for more acute clinical investigation than

the more vague symptoms of fatigue and sexual dysfunction that are common symptoms of hyperprolactinemia for males. After menopause, the symptoms of hyperprolactinemia in females are more similar to the constellation of symptoms seen in men and, consequently, the incidence is thereafter comparable. Due to mild clinical manifestations, the true incidence of lactotroph tumors can therefore be assumed to be underestimated in post-menopausal women and in men in general.[85]

Other hormone-producing PitNETs

For somatotroph, corticotroph, and thyrotroph tumors, the SIRs and corresponding proportions of total number of PitNETs, were comparable to previous publications.[25] There are, however, some methodological differences between our study and other epidemiological studies. In our study, data is only reported from adult patients, which would reasonably result in larger differences when comparing results with studies not excluding patients <18 years of age. In theory, this is most relevant in corticotroph and lactotroph tumors, which are the most common PitNETs in pre-pubertal and pubertal age groups, respectively.[86] Interestingly, no significant difference was seen in either the proportion or overall incidence in the results presented.

Only four patients were registered with thyrotroph tumors during study period. With so few cases, the SIR is to be interpreted with caution, as the statistic can change drastically with the addition or subtraction of even a single case. More reliable data is presented from a recently conducted incidence study that had 28 cases of TSH-producing tumors from the period 1990-2010, presenting an incidence of 0.15/1,000,000/year.[11] Only one case with an active gonadotropin-producing tumor was reported in our study.

Micro- and macroadenomas

We found macroadenomas to be more common than microadenomas (65% *vs* 33%, respectively, with 2% non-categorized). This proportion of macroadenomas is somewhat higher compared to other epidemiological studies, where macroadenomas are reported in the range from 41.3-56.9%.[23-29] This is also vastly different compared to the numbers presented in autopsy studies, where macroadenomas occur at a prevalence of 0.3-1%.[15,17] However, evidence suggests that tumors developing into macroadenomas grow fast and manifest clinically before any possible incidental discovery.[87] In radiologic material following patients with MRI-verified pituitary tumors for 3.5 and 10 years, no tumor growth was found in lesions <5 mm.[88] In macroadenomas, the growth rate was 20% and 50% at 3.5 and 10 years, respectively.[88] This means most small lesions do not gradually develop into larger tumors but stay small in most cases. Tumors with the potential to grow into macroadenomas will show rapid tumor growth and manifest clinically in most cases. This may explain the difference in macroadenoma prevalence in epidemiologic and post mortem studies.[89] However, with a smaller proportion of microadenomas in our study compared to other incidence studies, it is likely that there is an under-representation of microadenomas in our material. We hypothesize that this might be attributed to radiologic modalities with lower sensitivity for smaller lesions used during the study period in our region. To our knowledge, MRI scanners with capacity of 0.5-1.5 T have been the standard imaging modality for pituitary lesions in VGR during 2001-2011. In the last decade, 3-T MRI scanners, that demonstrate higher sensitivity for smaller lesions, have become more available and are now the standard modality for pituitary tumor assessment. Whether this progress in imaging quality has also resulted in an increased incidence for microadenomas is an interesting research topic for future study.

5.2 PAPERS II-III

Functional imaging and PET have developed rapidly in recent decades with an ever-growing plethora of new tracers for tumor detection. SSTR-PET has emerged as a cornerstone in NET imaging but, to our knowledge, ^{68}Ga-DOTATOC PET has not been previously studied in patients with PitNETs. The field of functional imaging for the detection of pituitary tumors is limited and there are even fewer studies evaluating PitNETs with the approach of SSTR imaging. As previously discussed in this thesis, several studies have evaluated PET tracers and SSTR scintigraphy with somewhat disappointing results. The aim in this thesis is to evaluate pre- and post-operative diagnostic properties of the tracer, ^{68}Ga-DOTATOC, which has shown promising results in PitNET-related tumors and also provides a better accessibility than most PET tracers.

Tumor uptake

This study evaluated tracer uptake in PitNETs that require surgical treatment in regard to their histopathological classification and SSTR expression. SUV_{max} was found to correlate with the expression intensity of SSTR2. For the other SSTR subtypes, no correlation was found with tracer uptake.

Thyrotroph tumors demonstrated the highest degree of tracer uptake, which was significantly higher than that of normal pituitary gland. This finding is of high clinical importance since the diagnostic procedure of thyrotroph tumors is complex and resource intense. The process of confirming a thyrotroph tumor is based on proving autonomous TSH production through extensive endocrine testing.[90] Also, since thyrotroph tumors in the pituitary are very rare and NF-PitNETs are much more common, diagnosis could not be determined even with MRI evidence of a pituitary tumor. Verified autonomous TSH production must be shown.[90] This thesis demonstrates that ^{68}Ga-DOTATOC-PET can be used

in this situation, since the tracer can distinguish between NF-PitNETs. Nonetheless, further research is still needed to confirm this conclusion more reliably since we only present a relatively low number of patients with thyrotroph tumors. This would probably entail a multinational study, as only 1-2 cases with a TSH-producing PitNET are diagnosed annually in Sweden.[11]

^{68}Ga-DOTATOC uptake stratified in PitNET subtypes by quantified SSTR expression is a new finding. We demonstrate significant differences in uptake in these subtypes, which may be of clinical importance. As discussed above, the tracer may be of great importance in distinguishing thyrotroph tumors from NF-PitNETs. Furthermore, high uptake of ^{68}Ga-DOTATOC might indicate which tumors could be potentially treated medically with SST analogues. In patients with acromegaly or a TSH-producing tumor, such SST analogue treatment is widely accepted but a considerable proportion of patients do not respond to this treatment. Also, in gonadotroph tumors, which are not traditionally treated with SST analogues, the tracer could potentially distinguish which patients might respond to medical SST treatment. For example, in our cohort, two of the seven gonadotroph tumors demonstrated high ^{68}Ga-DOTATOC uptake, which may indicate treatment response for pharmaceuticals targeting SSTR2. The other gonadotroph tumors in our cohort that demonstrated low tracer uptake expressed high levels of SSTR3, which could be of similar value for selecting SST analogues targeting this SSTR subtype. ^{68}Ga-DOTANOC is another gallium-labeled SST analogue, similar to DOTATOC, but demonstrates much higher affinity for the SSTR3 receptor (IC_{50} 40 nM).[91] Pasireotide is a SST analogue with a higher affinity for SSTR3.[92,93] SSTR-directed therapy is an interesting topic for future research.

Somatotroph tumors showed a medium-to-high median tracer uptake that is similar to the uptake demonstrated by the normal pituitary gland. The

difference was only statistically significant after 1 hour. It could be hypothesized, however, that ^{68}Ga-DOTATOC could still have some use in a dual-tracer approach with FDG-PET. In patients with acromegaly with uncertain tumor findings, FDG-PET has demonstrated elevated tracer uptake in contrast to normal pituitary tissue. However, FDG-PET only has a specificity of approximately 50%,[49] which makes positive uptake uncertain for finding a tumor. ^{68}Ga-DOTATOC uptake in somatotroph tumors cannot be reliably used to distinguish from normal pituitary tissue, but would help in discriminating these tumors from other etiologies with increased FDG uptake.

This dual-tracer approach was evaluated in corticotroph tumors in a recent publication by Wang and colleagues using a similar tracer to ours, ^{68}Ga-DOTATATE. They concluded that the sensitivity, specificity, and accuracy for the FDG/DOTATATE dual-tracer method was 88.9%, 96.3%, and 92.6%, respectively, compared to 70.4%, 85.2% and 77.8% for FDG-PET alone.[45] As in our study, corticotroph tumor uptake was generally lower compared to normal pituitary gland. Interestingly, we found higher median uptake in these tumors when using ^{68}Ga-DOTATOC, even though ^{68}Ga-DOTATATE demonstrates higher affinity to SSTR2.[91] However, further comparison is precarious since other methodological differences may also contribute. Even though both studies found significantly lower uptake in corticotroph tumors, the clinical significance is limited, especially in post-operative image assessment, as the lower uptake could not be concluded as a tumor-specific finding.

The tracer also has important clinical value by adding higher specificity for abnormal uptake, as incidental tracer uptake can be better explained. As FDG-PET steadily becomes used in clinical practice, the number of patients with incidental tracer uptake in the pituitary is also likely to increase. There are

some potential strengths with SSTR-PET over FDG-PET in this regard as the latter of these tracers has poor specificity for incidental uptake. Hyun and colleagues found PitNETs comprise only about 50% of the incidental FDG uptake in the pituitary gland.[49] Also in FDG-based studies, there seems to be no reason to report absolute SUV values because it could not distinguish reliably between diagnoses. In our study, we found a high ^{68}Ga-DOTATOC uptake to be specific for thyrotroph tumors. In addition, in somatotroph tumors, there was a medium-high uptake, which could help in distinguishing these tumors from corticotroph and non-functioning tumors. Although uptake was lower compared to the pituitary gland, there was a low-grade uptake in corticotroph tumors, which might be assumed to be higher than in most other pituitary incidental findings.

Histopathological data

A major strength of our study is that uptake data is accompanied by histopathological data. We show data regarding both cell type and SSTR expression as a basis for tracer uptake. A few attempts at characterizing SSTR profiles in PitNET tumors have been made in previous scintigraphic studies.[62-65] Lower number of tumors were assessed and the results were highly inconsistent in these studies. In addition, these studies showed results in a binary fashion, only presenting SSTR as present or absent. Further, no information regarding receptor subtype was presented. In our study, specific monoclonal antibodies towards SSTR1-3 and SSTR5 were used, allowing much more detailed and reliable description of SSTR expression.

SSTR2 and SSTR5 receptors were expressed to a high degree in somatotroph tumors. Paradoxically, these tumors demonstrated much lower tracer uptake compared to thyrotroph tumors despite both groups demonstrating equally high SSTR2 expression. Somatotroph tumors demonstrated much higher degrees of

both SSTR3 and SSTR5 expression, which paradoxically rendered a lower overall tracer uptake value. One hypothesis that could be derived from this is that an increased number of receptors induce competitive interference at the receptor level, which results in less receptor binding and consequently lower tracer uptake. Another explanation might be an inherent limitation in the IRS scale used to quantify SSTR expression levels. This scoring system presents values on an ordinal scale and based on visual, semi-quantitative determination. Thereby, there could still be a numerical or absolute difference in the number of receptors in these tumors, even though the staining intensity was optically determined as the highest score (IRS 12).

In total, three tumors demonstrated plurihormonal secretion. In the WHO 2017 classification of pituitary tumors, plurihormonal tumors are considered to be a distinct subgroup of tumors within the Pit1-positive cell lineage apart from lactotroph, somatotroph, and thyrotroph groups. However, to better correlate with clinical practice, these tumors were classified according to their clinical presentation on entering this study. Consequently, two plurihormonal tumors were added to the thyrotroph tumor group and one plurihormonal tumor to the somatotroph group. These plurihormonal tumors demonstrated no significant difference in uptake from the pure TSH-producing and somatotroph tumors and could not be considered as outliers to the groups to which they been added.

Post-operative assessment

Since most of the different types of PitNETs in our study showed lower or equally high uptake to the normal pituitary gland, ^{68}Ga-DOTATOC-PET generally added limited value to post-operative evaluation. Eleven patients showed an abnormal post-operative MRI with findings of either suspected residual tumor tissue or suspected scar tissue. In a clinical situation where no pre-operative ^{68}Ga-DOTATOC-PET has been performed, the tracer could only

accurately distinguish thyrotroph tumors by a characteristic high tumor uptake. This could, in theory, help to distinguish these tumors in post-operative imaging assessment. Unfortunately, there was only one patient with a thyrotroph tumor, who had an equivocal finding on the post-operative MRI scan. In this case, the post-operative tracer uptake demonstrated a far lower uptake compared to the pre-operative scan (Figure 7). The low uptake in this case strongly argued against residual tumor tissue and the patient was later deemed as clinically cured after further clinical investigation.

For other tumor types, tracer uptake could not be determined as a tumor-specific finding. Also, scar tissue demonstrated relatively high tracer uptake. However, for tumors with a pre-operative ^{68}Ga-DOTATOC-PET scan, comparison of uptake levels between the pre- and post-operative PET was useful for distinguishing clinical and biochemical remission from non-cure and cure.

6 CONCLUSION

Incidence study

In the largest target population to date, this study found an SIR of 3.9/100,000/year, which is higher compared to data presented for the same region from an earlier era. These results are important for the development of clinical guidelines and may assist in organizing and planning healthcare and medical resources.

Imaging study

We demonstrated a significant difference in tracer uptake between normal pituitary gland compared to thyrotroph, corticotroph, and gonadotroph tumors. A major strength of this study was that uptake data was accompanied by histopathological data on adenohypophyseal cell type and SSTR expression pattern for each tumor group.

We found that the tracer could be useful in post-operative imaging assessment when evaluating thyrotroph tumors. Also, for patients with a pre-operative ^{68}Ga-DOTATOC-PET scan with tumor uptake higher than the median uptake of the pituitary gland, we found that biochemical remission could be predicted if the post-operative uptake was <60% of the initial pre-operative uptake.

This study presents the histopathological basis for the differences in tracer uptake between different PitNETs subgroups. We also found a significant correlation between tracer uptake and expression of SSTR2, evoking the hypothesis that medical treatment response may be potentially monitored in tumors with excessive SSTR2 expression. Both these conclusions are interesting starting points for further more focused studies.

7 FUTURE PERSPECTIVES

The rapid evolution of MRI that we have seen during the 1990s has continued into the last decade. An ever-growing plethora of new imaging sequences provides better imaging conditions and higher tumor specificity. Ultra-high-field MRI at 7T and magnetospectroscopy (MRS) were, until recently, esoteric modalities reserved for research purposes but are now beginning to find clinical applications. Imaging at higher magnetic field strength provides increased spatial resolution, higher signal-to-noise ratio, and an ability to characterize tumor consistency. Better imaging resolution decreases volume-averaging effects, which mitigates the partial volume effect that is particularly deleterious when assessing smaller lesions. MRS adds functional information and can detect metabolites indicative of neoplasms. Both the improved imaging resolution by ultra-high-field scanners and the functional dimension provided by MRS, or a combination of the two, would benefit the imaging assessment for PitNETs in general and for microadenomas in particular. A few studies have evaluated such ultra-high-field scanners in a subset of PitNETs in a post-operative setting and the technique seems promising, providing spatial resolution high enough to detect lesion as small as 1 mm.[94-96] However, imaging with ultra-high magnetic fields does come with some challenges that might be difficult to overcome. As field strength increases so does the susceptibility for artifacts. The sellar region is particularly prone to MRI artifacts as the air, bone, and soft tissue surfaces in close proximity create perfect conditions for the appearance of artifacts. Likewise, these factors are also considered major limitations for the MRS technique and explain why the technique so far only have delivered convincing results in for large macroadenomas with supra-sellar extension.[97]

Despite impressive advances with MRI modalities, it thus seems that there are still some intrinsic difficulties for MRI imaging of the sellar region, a result of which PET will be maintained as a valuable modality for the foreseeable future. Moreover, PET has also demonstrated rapid development in recent years. High-resolution PET scanners are becoming more common, providing higher spatial resolution. Also, increased computer power and imaging processing steadily improves image quality, enabling more reliable assessment of smaller lesions.

Also, interesting new tracers have recently been introduced, that seems especially promising for PitNET imaging. A particularly interesting candidate for PitNET detection is the ^{18}F-fluoroethyltyrosine (FET) tracer which, like MET-PET, is able to visualize protein metabolism. Low FET uptake in normal pituitary tissue and adjacent sellar structures yields a high tumor-to-background contrast. In comparison to MET-PET, it has a longer half-life of approximately 120 minutes, which opens it up for broader clinical use as it is not limited by short half-life to an on-site cyclotron. Also, in contrast to both FDG- and MET-PET, the uptake in inflammatory lesions is low, which further increases the specificity and the potential use in post-operative settings.[98] This tracer has demonstrated impressive results in the detection of other brain tumors, mainly gliomas.[99] To my knowledge, this tracer has only been tested for PitNET detection in one recent case report,[100] but the result was promising and I expect to hear a lot more about this tracer in the near future.

Although it has often been claimed that MRI or PET has a methodological advantage over the other modality, there is no fundamental incompatibility between these two modalities. On the contrary, the information provided by each modality is complementary to other. Scanners that combine these two modalities, MRI-PET scanners, are beginning to be more available outside

research facilities. This combined hybrid modality has excellent prospects for PitNET evaluation. The high anatomical resolution by MRI and the tumor-specific tracer uptake by PET would be of immense benefit in the diagnostic work-up of PitNETs. It would also streamline the diagnostic process with less hospital visits for the patient and, hopefully, with the elimination of invasive procedures such as sinus petrosus catheterization. As these hybrid scanners become more available, we will hopefully see a convergence of the development in these two fields, discussed above, providing excellent imaging conditions for PitNETs, that could eventually bridge the gap between tumor detection and diagnosis.

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