New imaging strategies in neuroendocrine tumors

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Chapter 1

General introduction

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Neuroendocrine tumors comprise tumors, which originate from (neuro)endocrine cells throughout the body. They are rare tumors with a reported incidence of 5/100,000.[1] Neuroendocrine tumors can produce biogenic amines, peptides or proteins with endocrine activity, and are called functional neuroendocrine tumors if overproduction causes clinical symptoms. Neuroendocrine tumors can be divided in low-grade, intermediate-grade and high-grade tumors. This grading system depends on the mitotic count and Ki-67 index which reflect growth rate of the tumor. Low-grade and intermediate-grade are also called well-differentiated neuroendocrine tumors. In general, these tumors have a better prognosis than their high-grade counterparts.[2-3] In this thesis only well-differentiated neuroendocrine tumors have been addressed. Neuroendocrine tumors can occur sporadically, but can also be part of the hereditary tumor syndromes von Hippel-Lindau (VHL) disease and Multiple Endocrine Neoplasia type 1 (MEN1). In both tumor syndromes, patients are prone to develop pancreatic neuroendocrine tumors.

VHL patients have a germline mutation in the *VHL* tumor suppressor gene, located on the short arm of chromosome 3 (3p25-26).[4] A second hit in the corresponding gene leads to development of VHL-related manifestations. Loss of functional VHL protein can result in increased production of vascular endothelial growth factor A (VEGF-A). VEGF-A induces angiogenesis. The prevalence of VHL disease is about 1-2/100,000 with an estimated birth incidence of 1 per 36,000-43,000 live births.[5-6] Next to pancreatic neuroendocrine tumors, VHL patients are also at risk to develop hemangioblastomas in the central nervous system, endolymphatic sac tumors of the middle ear, pheochromocytomas, clear cell renal cell cancer, renal cysts and pancreatic cysts.[7] Mortality in VHL patients is often the result of metastasized renal cell cancer and neurological complications caused by (progressive) hemangioblastomas.[8-9] VHL patients have a shorter life expectancy compared to the general population.[10] Currently, no biomarkers are available that can predict disease progression.

In patients in whom VHL was diagnosed after 1990, life expectancy was increased with 16 years compared to VHL patients diagnosed before 1985.[10] This is probably the result of improved screening and treatment options in the last decades. An increased survival in VHL patients might result in an increasing prevalence of pancreatic neuroendocrine tumors.

MEN1 is caused by a mutation in the *MEN1* tumor suppressor gene located at chromosome 11 (11q13). The prevalence of MEN1 is 1-10/100,000.[11] In MEN1 patients, the prevalence of pancreatic neuroendocrine tumors is 30-75%.[11-12] In MEN1 both non-functional as well as functional pancreatic neuroendocrine tumors can occur, of which gastrinomas and insulinomas are most common. In addition to pancreatic neuroendocrine tumors, MEN1 patients are also prone to develop other tumors, including parathyroid and pituitary adenomas. In MEN1 patients, 33% of the mortality is MEN1-related, of which pancreatic neuroendocrine tumors are the leading cause of death.[13-15]

For localization of pancreatic neuroendocrine tumors, different anatomical and molecular imaging techniques are available. Anatomical imaging includes computed tomography (CT), magnetic resonance imaging (MRI), transabdominal ultrasound and endoscopic ultrasound (EUS). For molecular imaging tracers are available specific for neuroendocrine tumors, including somatostatin receptor scintigraphy (SRS). Positron emission tomography (PET) is available for pancreatic neuroendocrine tumors with the specific tracers 6-[F-18]fluoro-L-dihydrophenylalanin (^{18}F-DOPA) and ^{11}C-5-hydroytryptophan (^{11}C-5-HTP), based on the ability of amine precursor uptake and decarboxylation of neuroendocrine tumor cells. ^{11}C-5-HTP PET combined with CT is superior for detection of pancreatic neuroendocrine tumors in patients known with advanced disease.[16] Based on the available literature, EUS seems a promising method for early detection of pancreatic neuroendocrine tumors.[17]

Screening is recommended in both VHL disease and MEN1 for early tumor lesion detection. For screening of the kidneys, adrenal gland and pancreas, the international VHL guideline recommends a high quality transabdominal ultrasound every year and MRI at least every other year.[18] For pancreatic neuroendocrine tumor localization, the recently revised expert opinion based MEN1 guideline recommends MRI, CT or EUS once every year.[19] The earlier MEN1 guideline recommended MRI or CT, and SRS once every 3 years.[12] Unfortunately, the level of evidence of these recommendations is low. It is unknown which imaging technique is best for early detection of pancreatic neuroendocrine tumors in both VHL and MEN1 patients.

The only curative treatment of neuroendocrine tumors is surgery. Compared to epithelial tumors, neuroendocrine tumors often behave indolent, but can also act more aggressive and/or become resistant to treatment. Currently, more treatment options consisting of targeted agents are becoming available for patients with advanced/metastasized disease.[20] These are the consequences of the increasing knowledge on cell biological behavior of these tumors. Neuroendocrine tumors often are hypervascular. The VEGF-A receptor tyrosine kinase inhibitor sunitinib and the

mTOR inhibitor everolimus have beneficial effect in patients with advanced pancreatic neuroendocrine tumors.[21-22] Bevacizumab is an antibody which binds VEGF-A. Currently, VEGF-A can be imaged with PET by zirconium-89 (^{89}Zr) labeled bevacizumab. Imaging with ^{89}Zr-bevacizumab PET can potentially provide information about VEGF-A status at the tumor site non-invasively.

Aim of the thesis

The aim of this thesis is to evaluate EUS and ^{11}C-5-HTP PET for the early detection of pancreatic neuroendocrine tumors in VHL and MEN1 patients. Moreover, in VHL patients and patients with advanced progressive neuroendocrine tumors, we evaluated the feasibility of ^{89}Zr-bevacizumab PET to visualize VEGF-A in lesions.

Outline of the thesis

VHL disease is the only hereditary tumor syndrome with a high prevalence (~70%) of cysts located in the pancreas.[23] Using the monogenetic disorder VHL disease as a model might give insight in the pathophysiology of pancreatic cysts in general from a VHL point of view. In **chapter 2** we reviewed the literature to explain pancreatic cyst development. We searched the literature for *in vitro* and *in vivo* VHL models for (pancreatic) cyst development. PubMed search terms included von Hippel-Lindau and pancreatic cysts or cystadenoma, pancreatic serous cystic neoplasms, pancreatic neuroendocrine tumors or neoplasms, histopathology, *VHL*, pVHL, extracellular matrix, cytoskeleton and cilia. Due to the limited number articles of pancreatic cysts in VHL disease, no time restrictions were made. No studies could be retrieved on the role of *VHL* mutations in pancreatic cell lines, so we reviewed other VHL-related *in vitro* studies. Only articles in English were included. Relevant references from the selected articles were also reviewed.

In VHL patients the prevalence of pancreatic neuroendocrine tumors is 10-17%.[23-24] Unlike MEN1, only non-functional pancreatic neuroendocrine tumors occur in VHL disease. Next to pancreatic neuroendocrine tumors, pancreatic cysts occur in VHL, with some mimicking neuroendocrine tumors, since they can harbor a solid appearance on imaging. Since it is unknown which imaging method is best, the aim of the study reported in **chapter 3** was to evaluate the value of linear EUS and ^{11}C-5-HTP PET versus CT/MRI+ SRS for detection of pancreatic solid lesions suspected for neuroendocrine tumors in VHL patients. Eligible patients were those with genetically proven VHL or patients with clinically proven VHL with a 1st grade family

member with genetically confirmed VHL, with an age of ≥ 18 years. Excluded were pregnant patients and patients known with alcohol abuses and/or chronic pancreatitis. CT/MRI+ SRS were performed in patients' own center, before patients underwent linear EUS and ^{11}C-5-HTP PET at the University Medical Center of Groningen. Patient and lesion-based positivity for pancreatic solid lesions were calculated for all imaging techniques, by using the total outcome of the four imaging techniques as a composite reference standard.

Since evidence is limited for the best imaging method for MEN1 screening, we report in **chapter 4** a prospective study in MEN1 patients in which the value was assessed of linear EUS and ^{11}C-5-HTP-PET versus CT/MRI+ SRS for early detection of pancreatic lesions. Eligible were patients with ≥ 18 years of age, with genetically proven MEN1 or patients with clinically proven MEN1 with a 1st grade family member with genetically confirmed MEN1. CT/MRI+ SRS were performed in patients' own center, before patients underwent EUS and ^{11}C-5-HTP PET at the University Medical Center of Groningen. Patient and lesion-based positivity for pancreatic lesions were calculated for all imaging techniques, by using the total outcome of the four imaging techniques as a composite reference standard.

The *VHL* gene encodes for the VHL protein. The VHL protein is part of an E3-ubiquitin ligase complex in the cell, responsible for degradation of the α-subunit of the transcription factor hypoxia inducible factor (HIF) in normoxic conditions. During hypoxia, HIF-α will not be degraded, resulting in transport of HIF-α to the nucleus. Together with the ß-subunit HIF is formed, resulting in gene transcription that enhances cell survival. This includes expression of VEGF-A. If the VHL protein function is lost, HIF-α accumulates which subsequently can result in development of both benign and malignant lesions.[5]

Currently in VHL disease, no biomarkers are available to predict disease progression. Overexpression of VEGF-A has been shown in VHL-related hemangioblastomas and renal cell cancer.[25] Bevacizumab is an IgG1 antibody which binds VEGF-A. The aim of the study described in **chapter 5** was to assess if ^{89}Zr-bevacizumab PET can visualize manifestations in VHL patients, and if ^{89}Zr-bevacizumab uptake in non-malignant lesions can predict progression. Included were VHL patients known with hemangioblastomas located in the central nervous system, visualized on MRI. In addition to the recent VHL screening, a PET scan was performed 4 days after administration of the tracer ^{89}Zr-bevacizumab. VHL conventional screening was repeated within 12 months. ^{89}Zr-bevacizumab PET and MRI scans were fused in order to identify the specific VHL manifestations that were imaged with ^{89}Zr-bevacizumab PET. Lesion growth and/or presence of new VHL

manifestations were assessed and it was evaluated if ^{89}Zr-bevacizumab positive manifestations was associated with progressive disease based on conventional imaging and/or clinical progression.

More treatment options are becoming available for patients with advanced progressive neuroendocrine tumors.[20] Neuroendocrine tumors are hypervascular and VEGF-A is often present in tumor lesions.[26] In preclinical models, a down-stream effect of everolimus is VEGF-A inhibition.[27-28] Everolimus is effective in patients with advanced neuroendocrine tumors. [22, 29] Tumor VEGF-A production might lower during everolimus treatment in these patients. Therefore, in the feasibility study described in **chapter 6** we evaluated if tumor lesions can be visualized with ^{89}Zr-bevacizumab PET in patients with advanced progressive neuroendocrine tumors. Moreover, we assessed if ^{89}Zr-bevacizumab uptake in tumor lesions lowered after start of everolimus treatment. Included were patients > 18 years with well-differentiated neuroendocrine tumors progressive over the past year according to response evaluation criteria in solid tumors (RECIST) 1.1. At baseline, 2 weeks and 12 weeks a PET scan was performed 4 days after administration of the tracer ^{89}Zr-bevacizumab. After the baseline PET scan, everolimus therapy was started and continued until occurrence of progression of disease or intolerable toxicity. A CT scan was performed at baseline and every 3 months for response evaluation according to RECIST1.1. The number of visualized lesions on ^{89}Zr-bevacizumab PET was assessed and the maximum standardized uptake value (SUV_{max}) was calculated at baseline, 2 weeks and 12 weeks. Next to disease evaluation according to RECIST1.1, serum chromogranin A, serum VEGF-A and whole blood everolimus levels were assessed and correlated with ^{89}Zr-bevacizumab PET outcomes.

Chapter 7 gives a summary of the findings and addresses future perspectives and **chapter 8** gives a summary of this thesis in Dutch.

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