New imaging strategies in neuroendocrine tumors

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Chapter 7

Summary and future perspectives

Summary

Neuroendocrine tumors comprise tumors, which originate from (neuro)endocrine cells throughout the body. They are rare tumors with a reported incidence of 5 per 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. 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] This thesis focuses on well-differentiated neuroendocrine tumors.

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 may lead to development of VHL-related manifestations. Loss of functional VHL protein can result in increased production of vascular endothelial growth factor A (VEGF-A), which induces angiogenesis. 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.[5] VHL manifestations are often hypervascular lesions, which is likely a result of VEGF-A accumulation. Currently, no biomarkers are available that can predict disease progression. VHL patients have a shorter life expectancy compared to the general population.[6] In patients in whom VHL was diagnosed after 1990, life expectancy was increased with 16 years compared to VHL patients diagnosed before 1985.[6] This is probably the result of improved screening and treatment options in the last decades. The prevalence of pancreatic neuroendocrine tumors in VHL is about 10-17%.[7-9] A longer 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 per 100,000.[10] In MEN1 patients, the prevalence of pancreatic neuroendocrine tumors is 30-75%.[10-11] 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 is the leading cause of death.[12-14]

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.[15] For pancreatic neuroendocrine tumor localization, the recently revised expert opinion based MEN1 guideline recommends magnetic resonance imaging (MRI), computed tomography (CT) or endoscopic ultrasound (EUS) once every year.[16] The earlier MEN1 guideline recommended MRI or CT, and somatostatin receptor scintigraphy (SRS) once every 3 years.[11] 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 tumor syndromes.

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 locally advanced or metastasized disease.[17] These are the consequences of the increasing knowledge on cell biological behavior of these tumors. Neuroendocrine tumors are often hypervascular. The VEGF-A receptor tyrosine kinase inhibitor sunitinib and the mTOR inhibitor everolimus have a beneficial effect in patients with advanced pancreatic neuroendocrine tumors.[18-19] 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.

The aim of this thesis was to evaluate new imaging techniques for early detection of pancreatic manifestations in VHL and MEN1 patients. Moreover, in VHL patients and patients with advanced neuroendocrine tumors we evaluated the use ^{89}Zr-bevacizumab PET imaging to visualize VEGF-A in lesions.

Chapter 1 is the general introduction and outlines this thesis. In **chapter 2** we reviewed the existing literature to gather insight in pancreatic cyst development in VHL disease. Pancreatic cysts occur in ~70% of the VHL patients, making it the only hereditary tumor syndrome with such a high prevalence. Using VHL disease as a

model might give insight in the pathophysiology of pancreatic cysts in general. We searched the literature for *in vitro* and *in vivo* VHL models for (pancreatic) cyst development. An extensive PubMed search was performed with search terms including 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. Only articles in English were reviewed. Relevant references from the selected articles were also reviewed.

No studies could be retrieved concerning the role of *VHL* mutations in pancreatic cell lines, so we reviewed other VHL-related *in vitro* studies, which were mostly performed in renal cell cancer cell lines. Here, next to intracellular regulation of the transcription factor HIFα, pVHL has also stabilizing functions in the extracellular matrix and cytoskeleton.

To investigate the development of VHL-associated pancreatic manifestations *in vivo*, conditional mouse models have been generated using *Cre/Lox P* technology. In mice with *Vhlh* knock-out in pancreatic progenitor cells, pancreatic cysts were observed after 16-18 months. This is in contrast with another mouse model for cilia loss in pancreatic cells: the *Kif3a* conditional knock-out mouse model in pancreatic duct cells showed pancreatic cyst development already after a few days. These findings suggest that cilia loss in pancreatic duct cells is an important early event in pancreatic cyst development in general and in VHL disease. Based on the mouse models, histopathology and clinical findings, there are no indications that pancreatic neuroendocrine tumors and cysts in VHL are similar features. No malignant cysts are found in VHL and no clear relationship exists between presence of pancreatic cysts and pancreatic neuroendocrine tumors. In patients, pancreatic cysts in VHL disease often remain silent and are not associated with malignancy.

In VHL disease, the precise mortality rate due to pancreatic neuroendocrine tumors is not known. However, pancreatic neuroendocrine tumors have the potential to become malignant. A second well recognized problem is that differentiation between serous cyst adenomas and pancreatic neuroendocrine tumors on radiological imaging can be difficult. Therefore, sensitive, specific pancreatic neuroendocrine tumor imaging is warranted. In **chapter 3** we evaluated in a head-to-head comparison the value of EUS and ^{11}C-5-hydroxytryptophan (^{11}C-5-HTP) positron emission tomography (PET) versus standard screening in patients with VHL disease for early detection of pancreatic neuroendocrine tumors. Patients with a VHL mutation or with one VHL-manifestation and a mutation carrier as 1st grade family member were eligible. They were included following recent standard screening with abdominal CT or MRI, and SRS. They underwent EUS and ^{11}C-5-HTP PET. Patient and lesion-based

positivity for pancreatic solid lesions were calculated for all imaging techniques with composite reference standard. Patients were recruited from the university medical centers of Utrecht, Rotterdam and Groningen. Twenty-two patients were referred to the University Medical Center Groningen between February 2009 and August 2011. Ten patients (45%) had solid pancreatic lesions. At a patient-based level EUS was positive in ten, ^{11}C-5-HTP PET in one, CT/ MRI in seven, SRS in three and CT/ MRI+ SRS in seven patients. Twenty solid pancreatic lesions were detected: 18 with EUS, 3 with ^{11}C-5-HTP PET, 9 with CT/ MRI, 3 with SRS and 9 with CT/ MRI+ SRS. EUS found most lesions ($P < .05$) with a median size of 9.7 mm (range 2.9-55 mm). Most solid lesions were homogeneous, hypoechoic, iso-elastic and hypervascular based on power Doppler. Moreover, EUS found multiple pancreatic cysts in 18 patients with a median of 4 cysts (range 1-30) per patient. This study showed that EUS is the best imaging technique for pancreatic solid lesion detection in VHL patients. In this setting ^{11}C-5-HTP PET is of no value.

In **chapter 4** we aimed to perform a head-to-head comparison to evaluate the value of EUS and ^{11}C-5-HTP PET to detect pancreatic neuroendocrine tumors versus the recommended screening techniques in MEN1 patients. Patients with proven MEN1 mutation or with one MEN1-manifestation and a mutation carrier as 1st grade family member, with recent screening by abdominal CT or MRI, SRS and plasma/serum tumor markers, were eligible. Patients underwent EUS and ^{11}C-5-HTP PET. Patient- and lesion-based positivity was calculated for all imaging techniques. Patients were recruited from the university medical centers of Utrecht, Rotterdam, Nijmegen and Groningen. Forty-one patients were referred to the University Medical Center Groningen between February 2009 and August 2011. In 35 patients (85%), 108 pancreatic lesions were detected: EUS found 101 pancreatic lesions in 34 patients, ^{11}C-5-HTP PET 35 lesions in 19, and CT/MRI+ SRS 32 in 18. With EUS different lesion characteristics could be identified. Most lesions were hypoechoic and only 2% were hyperechoic. Of all lesions 15% were cystic, of which nine lesions had a thick wall. With elastography most lesions were classified as iso-elastic, but 24% showed rigidity. Finally, power Doppler showed hypervascularity in 42% of lesions. EUS detected more lesions compared to CT/MRI and SRS (alone or combined) ($P < .001$). In contrast, ^{11}C-5-HTP PET performed similar as CT and CT/MRI + SRS, but was superior compared to SRS alone in all lesions and for lesions > 1 cm ($P < .05$). This study showed in MEN1 patients EUS being superior for pancreatic solid lesion detection. Moreover, ^{11}C-5-HTP PET is of no value for early pancreatic lesion detection.

Currently in VHL disease, no biomarkers are available to predict disease activity or progression. In **chapter 5** we described a feasibility study with ^{89}Zr-bevacizumab PET in VHL patients. The primary objective was tumor visualization with PET and secondary whether lesion growth in VHL patients can be predicted by ^{89}Zr-bevacizumab PET. In total 22 VHL patients were included. ^{89}Zr-bevacizumab PET was able to visualize 59 lesions in 16 patients (73%) with a median maximum standardized uptake value (SUV_{max}) of 8.5 (range 1.3-35.8). Detection rate for lesions ≥ 10 mm was 30.8%. The majority of the PET positive lesions (85%) had a solid appearance. Nine out of 25 progressive lesions were visible on PET. This study showed, ^{89}Zr-bevacizumab PET is able to visualize VHL-associated lesions with a broad heterogeneity in tracer accumulation. However, ^{89}Zr-bevacizumab uptake does not predict lesion progression.

Everolimus (mTOR inhibitor) has proven to be effective in patients with neuroendocrine tumors. Currently, no companion diagnostics is available. In **chapter 6** we described a feasibility study with serial ^{89}Zr-bevacizumab PET scans in patients with advanced progressive neuroendocrine tumors receiving everolimus. PET scans were performed before, at 2 and 12 weeks of treatment. In four of the 14 patients who entered the study, no tumor lesions were visualized with ^{89}Zr-bevacizumab PET. In the remaining 10 (71%) patients, 19% of tumor lesions > 1 cm known from CT were visualized. Decrease of tumor SUV_{max} was present at 2 weeks (median -7%) (P = .09) and this further reduced at 12 weeks (median -35%) (P < .001). Δ SUV_{max} at 2 and 12 weeks correlated with % change on CT at 6 months (r^2 = 0.51, P < .05, r^2 = 0.61, P < .01 respectively). From this study, we can conclude that ^{89}Zr-bevacizumab PET visualizes tumor lesions in the majority of NET patients. Tumor uptake diminished during everolimus therapy, indicating that serial ^{89}Zr-bevacizumab PET might be useful as early predictive biomarker of anti-VEGF directed treatment in NET patients.

Future perspectives

In this thesis we have shown that EUS is superior for early pancreatic neuroendocrine tumor detection in MEN1 and VHL. Moreover, in VHL patients, disease manifestations can be visualized with ^{89}Zr-bevacizumab PET. In patients with advanced progressive neuroendocrine tumors, serial ^{89}Zr-bevacizumab tumor uptake reduces during everolimus therapy. Here, suggestions for implementation and

application are described for these new imaging techniques in the near future and for further research in this era.

EUS as new screening method in MEN1 and VHL disease

We showed excellent performance of EUS in MEN1 patients with pancreatic lesions. Therefore, this imaging strategy should be implemented in the MEN1 screening guideline as first choice imaging method instead of abdominal CT or MRI. This should be combined with multicenter studies to prove better clinical outcome due to implementation of EUS.

In MEN1, multiple pancreatic neuroendocrine tumors are a common manifestation with a prevalence of 30-75% and 80% in necropsy series.[11] Surgery should be considered if lesions exceed 1 cm.[16] However, the malignant potential of these tumors is unpredictable. Small pancreatic lesions (< 1 cm) can remain indolent for years in MEN1, as others can be progressive within a few months.[20] Next to allowing cytology by fine needle aspiration, surveillance with EUS permits meticulous follow-up of individual lesions, even the small ones. Probably, progressive lesions (lesions with a short doubling time) might be detected earlier. This can support decision making regarding surgery at an earlier stage since no tools are available in order to predict malignant potential, besides monitoring growth velocity. As pancreatic neuroendocrine tumors are the leading cause of death in MEN1,[14] this might increase survival in MEN1 patients. However, future multicenter follow-up studies are necessary to prove the value of EUS and the right moment to perform surgery in order to avoid advanced disease.

In our study in VHL patients, just as in MEN1, EUS also performed better compared to the other imaging techniques, but less convincing than in MEN1. Moreover, in pancreatic solid lesions > 1 cm, EUS and CT/ MRI performed similar in VHL patients. This may be explained by the fact that the hypervascularity in VHL neuroendocrine tumors might be more distinct, making it easier to visualize with CT/ MRI. However, EUS has an additional value in VHL in establishing growth velocity.

A hurdle in VHL patients can be the discrimination between neuroendocrine tumors and serous (microcystic) cystadenomas in the pancreas, since these cysts can mimic neuroendocrine tumors on anatomical imaging. The current anatomical imaging is not able to differentiate in 100% of the cases, including EUS. This makes pathological confirmation of a neuroendocrine tumor even more desirable, compared to MEN1. Fine needle aspiration of a pancreatic lesion can be performed during EUS in order to obtain cytology, but the cell yield in our studies in 16 patients was limited.

Another way to obtain tissue material with EUS is fine needle tissue acquisition.[21] With this method histological material can be obtained. In a recent prospective study, 30 patients with pancreatic lesions suspected of a sporadic neuroendocrine tumor underwent fine needle tissue acquisition with a 19 Gauge needle. In 93% of the patients with a lesion size ranging from 7-100 mm the diagnosis could be confirmed, without complications in all patients.[21] Possibly in MEN1 and VHL disease, fine needle tissue acquisition of pancreatic lesions could also improve the yield.

Next to monitoring and confirmation of pancreatic neuroendocrine tumors, predictors for growth and malignant potential are warranted in both MEN1 and VHL disease. Currently, size is the main predictor for malignancy. In addition, tumor doubling time is used in VHL patients as risk factor for malignancy.[15] Next to lesion size and growth rate, EUS characteristics might be useful to predict malignancy. In a retrospective EUS study, a univariate analysis of EUS findings showed that next to larger size, inhomogeneity and cystic changes are predictors of malignancy of sporadic pancreatic neuroendocrine tumors.[22] A disadvantage of this study was its retrospective setting. It might be of interest to collect prospectively EUS characteristics of pancreatic solid lesions in MEN1 and VHL disease in the search for tumor growth predictors. Examples of EUS characteristics besides inhomogeneity and cystic changes are hypervascularity, elasticity/ rigidity and echogenic appearance.

Another interesting approach to determine malignancy might be tumor DNA analysis. A recent paper showed the feasibility to obtain DNA material of 29 sporadic pancreatic neuroendocrine tumors by EUS-guided fine needle aspiration. In the malignant tumors (advanced disease) a higher number of chromosomal losses was found, compared to their benign counterparts.[22] This strategy might also be useful in MEN1 and VHL patients.

No role of ^{11}C-5-HTP PET for screening in MEN1 and VHL disease

^{11}C-5-HTP PET is not useful as screening tool for early detection, for ^{11}C-5-HTP PET detected only 32% of the pancreatic lesions in MEN1 and 15% of pancreatic solid lesions in VHL patients. Lack of detection is not only a result of limited resolution of PET, as in MEN1 ^{11}C-5-HTP PET detected only 51% of lesions > 1 cm. In an earlier study ^{11}C-5-HTP PET combined with CT showed superior imaging in patients with advanced pancreatic neuroendocrine tumors.[23] In comparison to our imaging studies in MEN1 and VHL patients with sporadically evidence for advanced disease, almost all patients in the advanced disease study had no hereditary tumor syndrome. These

differences in study populations might be an explanation for the limited performance of ^{11}C-5-HTP PET in MEN1 and VHL.

^{89}Zr-bevacizumab PET as biomarker in VHL disease

VHL protein (pVHL) loss is the first event in the development of benign or malignant lesions in VHL disease. Loss of pVHL may result in VEGF-A accumulation in VHL lesions, which can be visualized with ^{89}Zr-bevacizumab PET. Not all VHL-associated lesions were visualized with ^{89}Zr-bevacizumab PET. Moreover, in VHL lesions originating from both different and similar organs, a wide range of ^{89}Zr-bevacizumab uptake was present in visualized VHL lesions. This suggests heterogeneity in VEGF-A driven angiogenesis in the tumor microenvironment in VHL disease.

Next to usefulness as a prognostic biomarker, visualization with ^{89}Zr-bevacizumab PET might select VHL patients upfront who may benefit from VEGF-A targeted agents. These include the VEGF-A antibody bevacizumab, VEGF-A receptor tyrosine kinase inhibitor sunitinib and the mTOR inhibitor everolimus. In future studies, it is of interest to evaluate ^{89}Zr-bevacizumab PET in VHL patients with advanced disease eligible for anti-angiogenic therapy, in order to investigate if it can be used as a predictive marker for drug treatment efficacy.

^{89}Zr-bevacizumab PET as biomarker in patients with advanced neuroendocrine tumors treated with everolimus

The widely accepted method for tumor response to therapy is based on response evaluation criteria in solid tumors (RECIST) by using anatomical imaging. Compared to anatomical imaging, an advantage of molecular imaging might be that it provides insight in molecular tumor characteristics including those in the tumor microenvironment and potentially could serve a an early predictive biomarker.

In neuroendocrine tumors patients, four out of 14 patients had a negative baseline ^{89}Zr-bevacizumab PET scan. Patients with a negative baseline ^{89}Zr-bevacizumab PET might indicate that VEGF-A is not an important target for therapy. However, a negative baseline ^{89}Zr-bevacizumab PET scan did not indicate that everolimus treatment was not effective. This can be explained by the fact that everolimus does not only reduce tumor VEGF-A release, but has more anti-tumor effects. Current study suggests that in the near future molecular imaging might play a role in selecting the right treatment in patients with advanced neuroendocrine tumors.

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