Master Thesis Technical Medicine
Track Imaging & Intervention

INTRATUMORAL HOLMIUM MICROSPHERE INJECTION IN PANCREATIC CANCER

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Preface

In this master thesis I summarize the research from the past year in which I applied the curriculum of track Imaging and Intervention from the Master Technical Medicine. I performed this graduation thesis at the Radboud university medical center, at the department Nuclear Medicine, currently a subdivision of the department of Medical Imaging. This thesis is created in collaboration with the TU Delft, Faculty of Mechanical, Maritime and Materials Engineering (3mE). The content of this thesis is divided in three chapters: the general introduction, a development report, and the preliminary results of a pre-clinical trial. In the general introduction, the foundation of this thesis is explained. In the second chapter, '*Low-cost intratumoral injection device for holmium microspheres*' describes an accessory to holmium microspheres which is still used in ongoing studies. The third, '*Intratumoral holmium microsphere injection in ex-vivo pancreatic adenocarcinoma – preliminary results',* describes the first results of holmium microsphere injection in three human pancreatic adenocarcinomas.

After the main subjects, the future trends are discussed in the '*Future perspective*'. Finally, in the '*Reflection*', side projects are illustrated, and the graduation internship will be reflected upon. Writing this thesis and conducting the study were repeatedly challenging and fulfilled my purpose. Perhaps you may experience the same while reading it.

- Ysbrand Willink

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General introduction

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General introduction

This general introduction describes pancreatic cancer, the challenges of locally advanced pancreatic cancer (LAPC) and why holmium microspheres may play an important role in the future treatment of this disease.

Pancreatic cancer

Pancreatic cancer is an aggressive malignancy that manifests itself in the pancreas. The pancreas is an organ in the abdomen and is part of the digestive system. It is commonly known for its production of insulin and glucagon to maintain a correct blood sugar level and causing diabetes mellitus when this function fails. The pancreas also produces digestive juice to neutralize stomach acid and break down nutrients like carbohydrates, proteins, and fat. The incidence of pancreatic cancer has multiplied in the Netherlands since 1990 and now over 2700 new cases are reported each year.[1] Yearly diagnosis of pancreatic cancer is reported in up to 446 000 people on a global scale and almost 440 000 people die from the disease.[2] The similar incidence and mortality rates already suggest the poor prognosis of the disease. Pancreatic cancer holds one of the worst prognosis of all known malignancies with a 1-year survival of 20% and a 5-year-survival of 9%.[3] The main risk factors for pancreatic cancer are both type 1 and type 2 diabetes mellitus.[4] Cigarette smoking and obesity are the main influenceable risk factors for pancreatic cancer.[5] Other major non-genetic and proven risk factors include malnutrition, excessive alcohol consumption and excessive red meat intake.[6] It is estimated that only 5-10% of all pancreatic cancer is directly linked to a genetic alteration.[5]

The major cause of a bad prognosis for pancreatic cancer is when diagnosed at an advanced stage. This is due to the lack of early onset symptoms. Most symptoms that develop in an early-stage result from the tumor mass obstructing the common bile duct (ductus choledochus) or pancreatic duct (ductus pancreaticus) which supply bile and digestive juice to the duodenum and are located in the pancreatic head (see figure 1). Therefore, tumors in the pancreatic head are diagnosed more often (60-70%) and at an earlier stage.[7] When symptoms appear, patients often present themselves with abdominal pain, weight loss, steatorrhoea (excess fat in feces), new-onset diabetes or jaundice. If a tumor grows large enough it may also cause upper gastroduodenal obstruction.

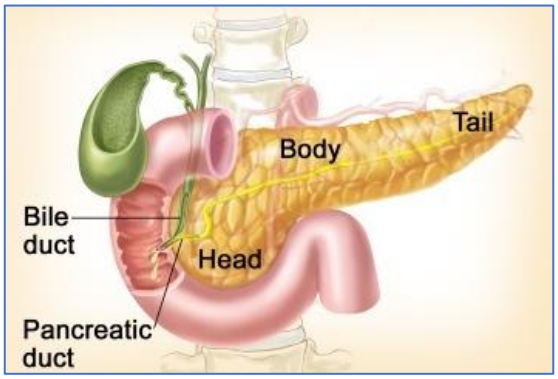

Figure 1 *Schematic overview of the pancreatic anatomy. Source: NCI, PDQ, Pancreatic Cancer Treatment.*

The primary imaging modality for identifying the stage, size, and burden of pancreatic cancer is computed tomography (CT). Both the arterial and venous phase needs to be included on the CT to assess possible vessel involvement. In larger medical centers and academic hospitals, magnetic resonance imaging (MRI) with contrast-agents may be used to further assess vessel involvement and metastasis. Also, magnetic resonance cholangiopancreatography (MRCP) may be performed by which the complete pancreaticobiliary tree, liver parenchyma and vascular structures can be imaged in three dimensions.[8] MRCP is reported to be as sensitive and specific (84% and 97%, respectively) in detecting pancreatic cancer as endoscopic retrograde cholangiopancreatography (ERCP), however, contrast does not need to be administered into the ductal system via endoscopy.[9]

If no distant metastasis was found on the imaging workup, commonly a transgastric or transduodenal fine-needle aspiration (FNA) biopsy is performed using endoscopic ultrasound (EUS). FNA by EUS may also be used to take samples of local lymph nodes to check for metastases. If a metastasis is found on radiological examination, only a biopsy of the metastasis is performed without further exploration of the primary tumor. The biopsy is used to identify the pathological origin of the tumor. The diagnostic workup when pancreatic cancer is suspected, is presented in figure 2.

The pathological origin of pancreatic cancer may arise from exocrine or endocrine parenchyma of the pancreas. In 95% of all pancreatic cancer, the origin is exocrine and in 80% it is identified as pancreatic adenocarcinoma. Therefore, the term pancreatic cancer often implies pancreatic adenocarcinoma, also known as pancreatic ductal adenocarcinoma. The macroscopic characteristics of pancreatic adenocarcinoma often include a hyperdense tumor which contains heterogeneous connective tissues and minimal vasculature. This creates a natural barrier against the perfusion of chemotherapy.[10]

The only current curative treatment option for pancreatic cancer is surgical resection. However, only 20% is eligible for primary surgical resection while the remaining patients suffer from locally advanced disease or distant metastasis at time of diagnosis.[11] Even when the tumor is identified as resectable by CT, in 26-68% of these cases the tumor is later found to be unresectable during surgical exploration.[12,13] After the pancreas is partially or completely resected, recurrence eventually occurs in 80%, resulting in a maximum 5-year survival of 25.2%.[14-18]

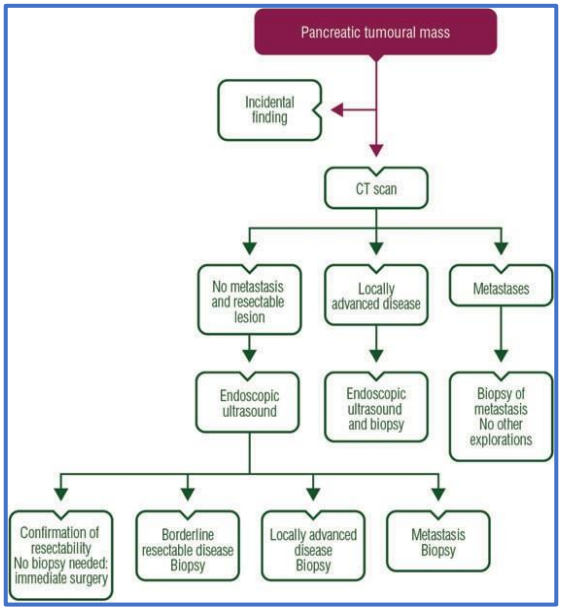

Figure 2 *Flow chart of the conventional diagnostic workup for a suspected pancreatic cancer. CT, Computed Tomography.*
Source: Ducreux, et al. ESMO guidelines, 2015.

Approximately halve of all patients with pancreatic cancer suffer from distant metastases (stage IV) at the time of diagnosis. This makes surgical resection obsolete since it does not increase survival.[19] Metastases often spread to nearby lymph nodes, the liver or the abdominal cavity. If the cancer has spread throughout the body and the patient is still vital, palliative chemotherapy can be started. The first choice of chemotherapy is FOLFIRINOX (a combination of 5-Fluorouracil, irinotecan, leucovorin and oxaliplatin) if the patient has a good general condition and low comorbidities. If the general condition is reduced and comorbidities present, nab-paclitaxel or gemcitabine may be used. Some patients are not eligible for any kind of chemotherapy and receive best-supportive care.[20] The follow-up of stage IV pancreatic cancer mainly focuses on pain-reduction and prolonging of survival. Although survival for stage IV pancreatic cancer is improved over the past decade, the 5-year survival remains at 5%.[21]

Locally advanced pancreatic cancer

Approximately 30% of the patients diagnosed with pancreatic cancer do not undergo surgical resection, even when distant metastasis is absent. This group suffers from locally advanced pancreatic cancer (LAPC). LAPC is commonly defined by the National Comprehensive Cancer Network (NCCN) guidelines as pancreatic cancer with >180 degrees arterial (superior mesenteric artery or Coeliac Axis) or aortic involvement or unreconstructable venous (superior mesenteric vein or portal vein) involvement without distant metastasis.[22] Not until recently, systemic chemotherapy with gemcitabine, with occasional additional radiotherapy, was the first-line therapy for LAPC and had median survival of approximately 12 months.[23] Current tumor control options for LAPC is mostly limited to systemic chemotherapy with FOLFIRINOX.[11] The best outcome when treating LAPC is down-staging followed by resection. A patient-level meta-analysis of FOLFIRINOX for LAPC reported a survival between 10.0 and 32.7 months, with a median survival of 24.2 months (95% CI: 21.6-26.8 months) from the start of the chemotherapy. However, selection bias was notable with 63.5% receiving radiation therapy, and 25.9% receiving post-chemotherapy resection.[24] Since systemic chemotherapy is very intensive, it is not an option for patients with a high comorbidity.[25] Despite enhanced chemotherapy schemes, disease progression eventually becomes inevitable.

Studies regarding local ablation techniques were increasingly conducted over the past decades. General ablation techniques that are based on thermal damage include radiofrequency ablation (RFA), freezing cryo-ablation and high intensity focused ultrasound (HIFU) and were tested in multiple

observational clinical trials. No large studies have been published which compare the effect of local ablation therapy to chemotherapy in patients with LAPC. Currently, nation-wide randomized controlled trials which combine chemotherapy and multiple local ablation techniques are being performed. Although some results seem promising, observational studies showed an increase in complication rates while the prognosis remained poor.[26] Furthermore, selection bias occurred in these studies. Patients who received ablation therapy, often successfully completed systemic chemotherapy without complications and without progressive disease. In the conclusions of ablation therapies, feasibility and safety is often granted. However, in absence of comparison studies, this statement may be invalidated. The most common forms of thermal ablation rely on placement of relatively large probes (17 Gauge = 1.15 mm) into the tumor. Ablation is identified as a minimally invasive therapy, however probe placement through healthy pancreatic tissue may lead to notable complications like pancreatic fistula, pancreatitis, and wound infection. Also, intestinal hemorrhages by thermal damage to blood vessels may occur. Thermal ablation techniques always use a peripheral safe zone to protect surrounding tissue from thermal damage. To reduce the risk of intestinal hemorrhages, this safe zone often includes vasculature which is encased by tumor cells in LAPC. This causes faster recurrence of the disease. However, some studies report that the increased immune response to the thermal damage may attack the remaining viable tumor cells as well.[27,28] Still, if surgical resection after down-staging is not possible, tumor recurrence is inevitable.

Although ablation gained popularity over the past decade, there are multiple techniques that do not rely on ablation to achieve tumor damage. Intratumoral therapies like immunotherapy, local chemotherapy, or brachytherapy may be used to achieve local tumor damage by different physical processes or biological pathways.[29-32] These therapies str further described and discussed in the literature review titled '*Intratumoral therapies for locally advanced pancreatic cancer – a systematic review*' (Annex A).

Although local treatment development sometimes seems promising, the prognosis of LAPC remains extremely poor, increased complications reduce quality of life, and down-staging resulting in resection remains rare. Local ablation techniques or other intratumoral therapies often lack therapy control and accuracy to establish down-staging. Tumor control and accurate targeting is essential to increase the treatment volume while preventing unwanted tissue damage. Therefore, a new or improved therapy for LAPC is urgently needed and further research is necessary.

Holmium microspheres

Holmium (Ho) is a chemical element that cannot be found as a free element in nature. When isolated, the stable isotope holmium-165 (^{165}Ho) can be activated by neutron bombardment in a nuclear reactor into a radioactive isotope. This is called neutron irradiation (n, γ). When ^{165}Ho is activated, it gains a neutron and the unstable isotope ^{166}Ho is formed. This is the most common method to produce ^{166}Ho. High energy beta particles are emitted from ^{166}Ho with a maximum energy of 1.85 MeV. In addition, low-energy gamma photons (yield of 6.7%) are emitted with a mean energy of 80.6 KeV.[33] The half-life of ^{166}Ho is 26.83 hours and thus, over 90% of the initial activity is released after 4 days and almost 99% is released after a week. After the decay, the stable and non-reactive isotope Erbium-166 (^{166}Er) is formed (see figure 3).

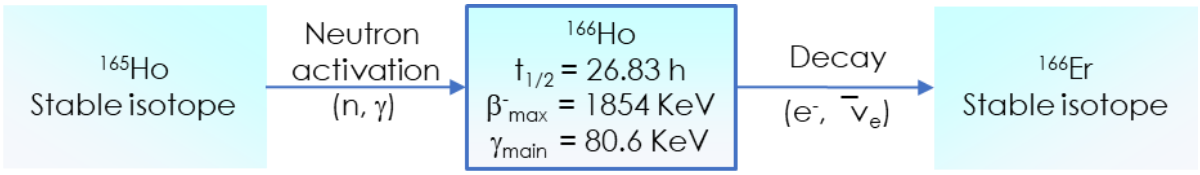

Figure 3 *Neutron activation scheme of Holmium-165 (^{165}Ho) to radioactive Holmium-166 (^{166}Ho) and its decay to stable Erbium-166 (^{166}Er) by beta-minus (β^-) emission.*

The medical application of ^{166}Ho was first described by Mumper, et al. (1991) who incorporated the isotope in microspheres to study selective internal radiation therapy (SIRT) of hepatic tumors.[34] SIRT is also commonly known as transarterial radioembolization (TARE). The first microspheres incorporating holmium were made of acetylacetonate-poly(L-lactic acid). The production of these microspheres was altered and optimized using a solvent evaporation method. Eventually, biocompatible holmium poly(L-lactic acid) microspheres (Ho-PLLA-MS, HoMS) were produced in compliance with the European Pharmacopoeia.[35] The morphological shape and concentration of holmium in the microspheres would remain stable after neutron activation and long after releasing its energy (270 hours).[35] The microspheres have a diameter between 15 and 60 micrometer (µm) and a mean diameter of 30 µm (± 5 µm). Before nuclear activation, the microspheres contain 17-20% (w/w) ^{165}Ho which results in a specific activity of 5-10 MBq/mg Ho-PLLA-MS, depending on the desired activity at time of treatment. The specific activity may be increased even further for research purposes. The beta-particles emitting from the holmium in the microspheres can penetrate soft tissue up to 8.7 mm with a mean distance of 2.5 mm. In 2005 Ho-PLLA-MS received the CE-mark for SIRT of hepatic tumors under the brand name QuiremSpheres®. Since then, studies regarding treatment optimization and expansion of the intended use have been conducted.[36]

Beta-particles emitted from the ^{166}HoMS cause a very local high intensity radiation dose, while saving surrounding tissues. Besides the beta-particles, the low-energy gamma radiation can be used for therapy quantification using single photon emission computed tomography (SPECT). HoMS are visible on CT due to the high density of the holmium causing strong attenuation. Recent studies even performed HoMS quantification using unenhanced CT in phantoms, tumor bearing rabbits and humans.[37] Even magnetic resonance imaging (MRI) may be used to estimate the microsphere distribution and the subsequent dose distribution due to the paramagnetic properties of holmium.[38] In technical terms: the specific signal relaxation time is identified for each voxel using mono-exponential fitting algorithms over multiple echo times (multi gradient echo; MGE). By applying this fitting algorithm over all signals of the different echo times, a relaxation map (R_2^*) is constructed. By subtracting the average value of the pre-injection (or embolization) R_2^* maps from the post-injection R_2^* maps, and comparing with known scanner specific calibration values, the holmium concentration (mg/ml) can be determined. By adding the specific activity (MBq/mg) to this equation, the dose distribution can be calculated.

After successfully implementing HoMS for SIRT on hepatic cancer, new advantages of HoMS over conventional therapies were studied.[36] The vasculature in hepatic cancer is unique for the liver and SIRT becomes less effective in less perfused tumors. Another method to implant HoMS into a tumor is by direct intratumoral injection. Intratumoral injection of HoMS was first tested in animal studies. HoMS were injected in an *in-vivo* setting in mice with renal cell carcinoma, rabbits with induced squamous cell carcinoma, and feline patients with hepatocellular carcinoma. These studies already showed high tumor-absorbed dose without major adverse events.[39-41] Thereafter, the safety and efficacy of ^{166}Ho injection was evaluated in 13 feline patients with spontaneous unresectable squamous cell carcinoma. Although the intratumoral distribution of the microspheres was sub-optimal, local response was achieved in 55% of the patients.[42] Finally, the feasibility of direct intratumoral injection of HoMS was tested on human patients suffering from locoregional recurrences of squamous cell carcinoma in the head and neck region. Injection was performed under ultrasound guidance in three patients. Again, intratumoral dose distribution was suboptimal. Intratumoral distribution problems were caused by technical difficulties like microsphere accumulation and high intratumoral pressure. Still, no adverse events were experienced, although therapeutic effects were minimal.[43] This proved the concept of intratumoral injection of HoMS. Development of an intratumoral injection device of HoMS and treatment optimization may result in a safe, effective, and traceable therapy for solid tumors. This thesis describes the pre-clinical optimization of intratumoral HoMS injection in human pancreatic cancer.

Research objectives

The long-term goal of this research is to improve survival and quality of life of patients suffering from pancreatic cancer. This goal may be achieved by developing a new treatment method using holmium microspheres for direct injection in patients suffering from LAPC. The research objectives are:

1. Developing an intratumoral injection device for holmium microspheres to overcome previously found limitations, and
2. Describing preliminary results from the first injections of holmium microspheres in *ex-vivo* pancreatic cancer

Chapter 1: Low-cost intratumoral injection device for holmium microspheres

The main objective of this chapter is to develop and describe a device that could inject a homogeneous holmium suspension into solid tumors. Described requirements include suspension homogenization, suspension injection control, patient safety, operator safety, usability, cost, and availability.

Chapter 2: Intratumoral injection of holmium microspheres in *ex-vivo* pancreatic adenocarcinoma

This chapter shows the injection device during the first injections in human pancreatic cancer. The goal of this chapter is to test feasibility of holmium microsphere injection into pancreatic adenocarcinoma after surgical resection. Since this is the last pre-clinical study before advancing to a clinical trial, the results are essential for the following pilot-study in patients with pancreatic cancer: '*Intratumoral holmium microspheres brachytherapy for patient with pancreatic cancer; a single center, non-randomized, feasibility study in an open surgical setting – the SLOTH1 study*'.

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Low-cost intratumoral injection device for holmium microspheres

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Low-cost intratumoral injection device for holmium microspheres

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Abstract

Introduction: Intratumoral injection of holmium poly(L-lactic acid) microspheres (Ho-PLLA-MS) showed limited distribution throughout squamous cell carcinoma in previous studies.[2, 3] An important cause was agglomeration of microspheres in their suspension, resulting in heterogeneous injection. Agglomeration also limited dose predictability and microsphere control. The aim of this project was to develop a quickly applicable intratumoral injection device for suspension homogenization and improved intratumoral dose distribution.

Development: Manual rotation over the long axis of a syringe containing the holmium microsphere suspension was the desired method of homogenization. Only CE-certified components were used and assembled with Luer-locks for high pressure resistance. A combination of two stopcocks created an axis on which a 3 ml or 5 ml syringe with the main suspension could rotate. The stopcocks also created a 90-degree angle between the main syringe and an injection needle to overcome injection angle limitations while the syringe remained in a horizontal position for optimal homogenization. The device contained a dead volume, in which the suspension cannot be controlled, of just 0.24 ml.

Testing: First, maximum deviation from the average microsphere concentration of multiple injections was validated in a non-clinical setting. Out of a 5 ml syringe with a non-radioactive Ho-PLLA-MS concentration of 50 mg per 1 ml of 0.1% Pluronic, 5 deposits of 1 ml were injected in a vial. This was repeated using two 3 ml syringes, with 15 mg per 1 ml of 0.1% Pluronic, which made 9 deposits of 0.5 ml. The holmium microsphere and Pluronic residue was weighed after evaporation to estimate the concentration per vial. The maximum deviation from the average concentration was -10,3% to 5.4% and -5.8% to 3.0% for the 5 ml syringe (average 59.9 mg/ml, SD 3.7 mg/ml) and 3 ml syringe (15.9 mg/ml, SD 0.42 mg/ml), respectively.

Thereafter, a canine patient suffering from a solid, unresectable anal sac carcinoma of 175 cm^3 was treated using the rotation device. Under CT-guidance radioactive Ho-PLLA-MS injection was performed. From the 3.72 GBq prepared in six, 5 ml syringes, 2.84 GBq (77%) was injected. The remaining 23% was lost in the syringes (8%), stopcocks (13%) and absorbing mats/gauze (2%). This resulted in a maximum absorbed tumor dose of 258 Gy. CT and MRI showed a clear dose distribution throughout the tumor. A lung dose of approximately 400 MBq (14.1%) was measured on SPECT imaging. However, the dog did not suffer any complications. After 6 weeks, the tumor had reduced to 125 cm^3 (28.6% volume reduction) and was successfully removed by surgical intervention.

Discussion: The first prototype of an intratumoral injection device for holmium microspheres showed promising results. Suspension can be homogenized up to 10.3% by manual rotation of a 5 ml or 3 ml syringe. The canine's anal sac carcinoma had a similar high-density structure as seen in human pancreatic cancer and was therefore suitable for a simulation experiment. Although homogenization was successful, performance of the device was still dependent on many human factors. Future improvements may include automatic syringe rotation, remote injection, injection volume limiters and high viscous suspensions.

Conclusion: The intratumoral injection device for holmium microspheres was successfully validated and tested for the first time on a canine patient. The device is feasible and safe to use in future trials.

Introduction

Holmium microspheres (HoMS) were originally developed for selective internal radiation therapy (SIRT) of hepatic tumors. This treatment method cannot be directly translated to application in pancreatic cancer.[1] For SIRT, a predetermined amount of HoMS is implanted in hepatic tumors via an arterial catheter. A lower scout dose is used to evaluate HoMS distribution to other organs or healthy liver parenchyma. Although this method also has its limitations, like potential shunting to the lungs, undesired dose to healthy liver parenchyma, and possible coil embolization, all HoMS are simply released in the predetermined liver artery.[2] The catheter is then flushed with saline to release any agglomerated microspheres. The microspheres lodge in the small capillary of the tumor, where they release their energy and damage the tumor. The goal is to deliver a high tumor dose via the dominant arterial blood supply of the tumor. The liver parenchyma is exposed to a lower dose since it receives 75% of its blood supply via the portal vein.[3] Since solid pancreatic cancers have a very limited perfusion and the pancreas only receive blood from the arteries, SIRT with HoMS is not an optimal treatment method. Multiple studies have described other methods for intratumoral therapy delivery in patients with pancreatic cancer. These studies have been summarized in a systematic review (see Annex A). One method, which is currently also being applied for micro brachytherapy of phosphorus-32 microparticles, is direct intratumoral injection.[4,5] Possibly, a similar treatment method can be developed for intratumoral injection of HoMS.

To deliver HoMS by direct intratumoral injection into pancreatic cancer, new limitations must be solved. Since the application of this novel treatment method was only published twice, this method is relatively new and not extensively investigated.[6,7] The first publication injected radioactive HoMS in squamous cell tumors in feline patients with oral tumors three years ago, and the second on human patients with recurrent head and neck tumors two years ago. Both studies performed intratumoral injection of HoMS using 1 ml prefilled syringes. The syringes were connected to 21 Gauge (G, 0.819 mm) or 23G (0.641 mm) hypodermic needles using Luer-lock connections. The HoMS were suspended in a 2.0% Pluronic (F-68, Sigma-Aldrich Chemie B.V., Zwijndrecht, The Netherlands) which functioned as a carrier for the HoMS. Gentle agitation or shaking of the prefilled syringes was used to provide a visually homogeneous suspension right before injection. The syringes were secured in a polymethyl methacrylate (acrylic) cover to protect the operator from the beta radiation.

In the first study by van Nimwegen et al. (2017)[6], 13 feline patients suffering from spontaneous unresectable squamous cell carcinoma were injected with ^{166}Ho-PLLA-MS. Each syringe was used to deliver 2-3 deposits, which were separated by ≤6 mm to ensure homogeneous dose distribution throughout the tumor and reduce the chance of large deposits in healthy tissue or vasculature. Because all tumors were located on the tongue or mandibles, the injections were visually performed without image guidance. Each treatment contained a total quantity of 200 mg HoMS in 30-60% of the tumor volume. On average, 59.8% (SD = 17.6%) of the prepared activity in the syringes, was administered into the tumors. The remaining 40.2% was lost in the syringes, needles, or leaked back through the needle tract. Three dose cohorts were used with a median absorbed dose of 547 Gy (range: 81 – 4162 Gy) with minimal side-effects. One patient suffered from a local radiation ulcer, probably due to an injection in healthy tissue. Although small deposits were used, two patients received >20% of the initial tumor dose in the lung region and one patient died. Although unlikely, artery embolization in the lungs after accidental intravenous injection could not be completely dismissed. Regarding tumor shrinkage, responders showed a significant smaller initial tumor volume (1.9 ± 1.0 cm^3) than non-responders (4.2 ± 1.7 cm^3, $p<.05$). This was most likely related to the microsphere distribution, and thus the dose-distribution throughout the tumor. Especially in larger tumors it is difficult to obtain a homogeneous dose-distribution because of the limited penetration depth of the beta particles.

In a following study by Bakker et al. (2018)[7], three human patients with locoregional recurrence of head and neck squamous cell carcinoma received intratumoral injection of radioactive HoMS. Intratumoral injections were performed using ultrasound guidance. In the initial planning, two patients would receive 0.5 ml suspension with 100 mg of HoMS and one

patient would receive 250 mg in 0.2 ml suspension. Although 50% of the prepared activity in the syringes was expected to be administered, 84.3%, 9.5% and 79.2% were injected in patient 1, 2 and 3, respectively. In patient 2, a needle obstruction occurred almost immediately, and the procedure was aborted. The obstruction was most likely caused by the high intratumoral pressure. In the other patients, some leakage from the tumors occurred. Also a few tumors were filled with necrotic fluid. This caused precipitation on the bottom of the tumor. No major side effects occurred in any patient, and no activity was seen outside the tumors on post-injection imaging.

Optimization of intratumoral spatial distribution of the HoMS was strongly advised by both studies.[6,7] A critical causal factor causing the suboptimal distribution was the heterogeneous suspension. This caused unpredictable fluctuation in microsphere concentrations between injections. The aim of this project was to analyze the limitations of intratumoral HoMS injection for pancreatic cancer and to develop an immediate applicable intratumoral injection device with increased suspension homogenization to improve intratumoral HoMS distribution.

Problem-analysis

Problem-analysis

The HoMS showed to be difficult to distribute homogeneously throughout the tumorous tissue. This occurred in both solid tumors and cyst-like tumors.[6,7] This project only focuses on solid pancreatic cancer. In the first stage of intratumoral injection the fluid functions as a carrier for the microspheres. When the injection is performed, the microspheres most likely agglomerate in the tumor and the fluid is either absorbed or remains in place. The suspension and microspheres can be controlled up to the moment of injection. Within the tumor, the suspension is only controlled by gravity, tumor pressure and injection pressure. How the suspension is injected, e.g., concentration, flow, location, and volume, may be controlled by the operator and need to be predetermined. It is however unlikely that a standardized injection protocol can be used for all tumors, since tumors are highly heterogeneous and variate between patients in size, density, and structure.

Physical effects

The physical occurrence that caused the most limitations in previous studies, was the sedimentation of the HoMS in the fluid. The sinking and agglomeration on the bottom of the vial or syringe is caused by the difference in density between the HoMS and the fluid. HoMS has a relatively high density, of 1.4 g/cm^3, due to the 18.8% (w/w) of holmium inside.[1,8] The fluid density is approximately 1.0 g/cm^3. The fluid previously used consists of 2.0% Pluronic and could be further diluted with 0.9% NaCl (saline). Pluronic is a non-ionic surfactant which causes the water in the suspension to lose its surface tension which inhibits shear forces. Surface tension and shear force make microspheres float or stick to the sides of the vial or syringe. Saline is used to create an osmotic equilibrium inside and outside the cells. When ignoring drag by friction, shear force or surface tension, this results in an estimated acceleration of the microspheres to the bottom of any container of almost 2.75 m/s^2 (see Appendix I). To resolve sedimentation and agglomeration, gentle agitation was used in previous studies. However, due to fast sedimentation, within seconds after agitation the first sediment forms. Shaking of the container may cause air bubbles to form and increase the risk of radioactive spillage from the syringe. Therefore, a new method to create a homogeneous suspension of the microspheres in their carrier needs to be developed so a controlled injection can be performed.

Dead volume

A complementary problem that causes inhomogeneous injection of the microspheres is the so-called dead volume. The dead volume is the inner volume, or lumen, of any syringe, needle, three-way stopcock, or tubing that is out of control for the operator. As mentioned above, the total volume injected is separated over multiple smaller deposits which in previous studies varied from 0.1 to 0.5 ml.[6,7] Should the dead volume of an injection device have a volume larger than 0.5 ml, which is not uncommon, microsphere concentration in small deposits could vary severely and injection concentrations becomes unpredictable. In theory, a smaller dead volume should correlate with less variation in microsphere administration between injections.

Legal restrictions

The Medical Device Regulations (MDR) of the European Union also have an important role in this project. Since this device is an accessory to the HoMS, which is a medical implantation device, it needs to meet the MDR regulations. The device needed to be operational within 3 months for experimental use and within a year for patient use, and thus independent certification was not an option. This limits material use, fabrication options, and experimental use of components.

Summarized problem-analysis

To summarize, control over the HoMS concentration in suspension is shortly available during motion the main suspension container. Relatively heavy microspheres sink within seconds and cannot be controlled within the so-called dead volume of a device. Components that have direct contact with the suspension are limited to already CE certified components which cannot be applied outside their intended use. The aim was to develop an injection device within the limitations stated above, that could dispense a homogeneous microsphere suspension with a maximum variation of 20% from average in a pre-clinical setting.

Injection device development

Homogenization methods

First, the preferred method of homogenization needed to be established. To keep the microspheres in a homogeneous suspension, two methods could be applied. The first is to create a balance between the gravitational pull on the microspheres (downward force) and their buoyancy (upward force) in the suspension. This balance can be achieved by either reducing the microsphere density or by increasing the fluid density. Another method to achieve balance is by adding a third force in the equation, viscosity. When increasing the viscosity of the fluid, all movement is getting inhibited. Because the microspheres are a registered and certified medical device that contain a relatively heavy component, namely holmium, reducing the microsphere density, is unlikely. To adjust the density or viscosity of the fluid, new components need to be added and new biocompatibility and radiation effects need to be studied extensively. Microcrystalline cellulose (MCC) has a higher viscosity due to a network of cellulose chains within the suspension and is currently under investigation for biocompatibility.

Continuous motion

The second option to keep the microspheres homogeneous in suspension is by continuous motion. Similar to gentle agitation in previous studies, continuous movement could homogenize the suspension indefinitely.[6,7] Suspension movement must be performed up to the moment of injecting the suspension, instead of up to the moment of needle placement. The method of HoMS distribution throughout the suspension mimics the event of molecular diffusion, only on a larger scale. By continuous motion and random contact with other microspheres the microspheres are pushed towards the part with the lowest concentration. However, due to their high density the microspheres cannot diffuse against gravity and need to be kept in motion with their suspension. Due to the relatively simple solution and many applicable methods, homogenization by continuous motion was established as method of homogenization.

Materials

The purpose of this device was to be used in a feasibility study, which would not result in direct sale or marketing of this device. Therefore, extensive MDR certification could be evaded. This still required that already CE certified components are only used within their intended use and other components are only fabricated in-house. To minimize expenses, it was preferred to only use disposable components, already commonly used in the Department of Nuclear Medicine.

Syringes

Since syringes are CE certified, available in many shapes, sizes, connection parts and volume scales, they form the basis of the injection system as the main container of the microsphere suspension. A rotational motion in the length of a syringe was applied to keep the suspension visually homogeneous, while the tip and plunger of the syringe stay in place (see figure 1). Total volume, accuracy of volume indication and usability depends on syringe size and type. It was established that the best volumes for intratumoral injection were 3 or 5 ml (see Appendix II). Visual agglomeration occurred faster in smaller syringes. Visual agglomeration of HoMS in a 1 ml syringe

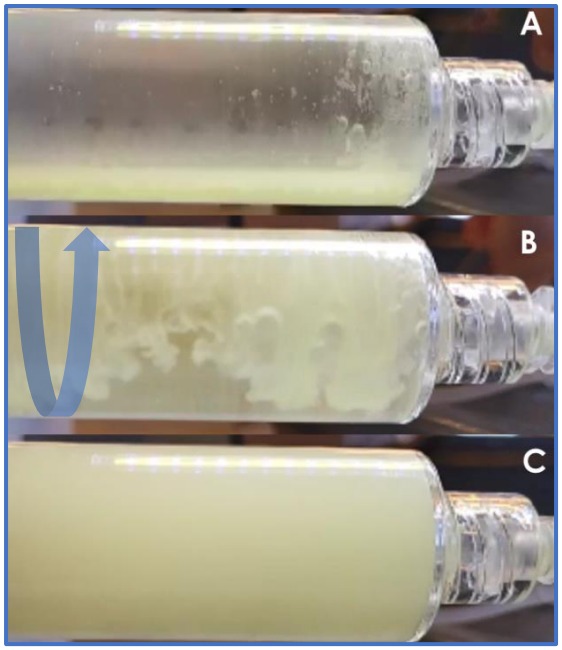

Figure 1 *Homogenization of holmium microsphere suspension by continuous rotation over the horizontal axis of a 10 ml syringe. A: Microsphere sediment on the bottom of the syringe before rotation. B: falling microspheres during rotation. C: visual homogeneous suspension after 8 rotations.*

occurred within one second. A smaller syringe volume than 5 ml was suggested by the operator after first intratumoral testing (see '*First application of a new intratumoral injection device for holmium microspheres in a canine patient*'). A smaller syringe increases haptic feedback from the intratumoral pressure on the plunger and can dispense volumes and control flow more accurately. Larger syringes were not feasible since larger volumes are not required for intratumoral injection in humans. Also, the described volumes become less accurate and larger plungers cause loss of haptic feedback for the operator.

Achieving homogenization by rotation

Visual homogenization of a HoMS suspension was only achieved when the syringe was held horizontally to prevent agglomeration in the tip or at the plunger of the syringe. However, to increase injection angles and usability, the needle should be able to approach the target both horizontally and vertically. An angle of 90 degrees between the syringe and needle, combined with complete 360-degrees rotational freedom of the syringe was required to inject under all angles while the syringe remained horizontal. Multiple combinations of different rotational and extension components have been assembled and tested for functionality, usability, dead volume, availability, and price. Additional requirements were Luer-lock connections, sterile delivery and disposable. A combination of two, three-way stopcocks (Codan) with each a 360° rotating male Luer-lock connection and two fixed female Luer-lock connections (see Appendix II) was chosen as the optimal solution. The three-way stopcocks were already in use in the department of Nuclear Medicine and had a combined dead volume of just 0.24 ml. Female-female and male-male Luer-lock connections were excluded due to their additional dead volume of approximately 0.25 ml and 0.12 ml, respectively. No other components, or combination of components had rotating parts for the syringe or a 90-degree angle for the needle. Further exclusion of components was based on price and availability. A used component overview is shown in Appendix II.

Feasible needles

Since needle blockage could not be correlated to a needle diameter at 21 G (0.819 mm, dead volume = 0.05 ml) in previous studies, this was chosen as smallest feasible needle for testing (see Appendix II).[6,7] If more tissue is damaged by needle penetration, the changes of infection increase with it. Therefore, no larger needles than 21G were advised.

First prototype

This section described the development of the first prototype for the holmium microsphere injection device (see figure 2.) The combined dead volume of the device seen in figure 2 is 0.3

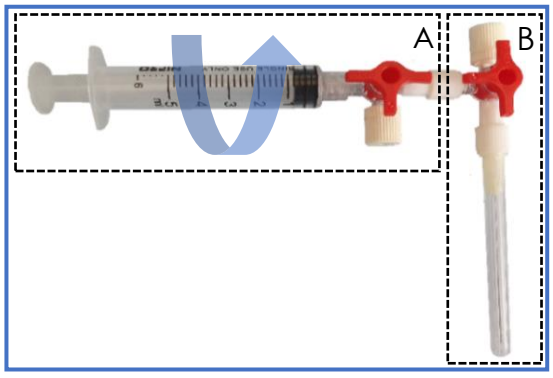

Figure 2 *Simplified injection device using two 360-degree rotatable stopcocks for suspension homogenization. Rotation occurs on the plane between section A and B. Total dead volume is 0.3 ml. A. The syringe and stopcock can rotate 360° in both directions. B. Needle in 90° which may be stabilized by an extension on the*

ml. The prototype was used for further pre-clinical and veterinary testing. The testing, validation, and further development of the device to meet all stated requirements is performed over all following investigations of this thesis. An overview of the device requirements and solutions is presented in Appendix III. The goal was to test the feasibility of the design and optimize the performance, safety, and usability.

Pre-clinical validation

Method

To validate the homogeneous dispensing ability of the injection device a suspension of 50 mg ^{165}Ho-PLLA-MS (non-radioactive) per 1 ml 0.1% Pluronic was drawn into a 5 ml syringe. The syringe was maximally filled, past the 5ml mark. While holding the syringe horizontal, the suspension was homogenized by rotating the syringe by hand, until visually homogeneous (see figure 1) with an additional 10 full rotations. Directly after rotating, the system was flushed, and the syringe was calibrated to the 5 ml mark. Next, the syringe was emptied by dispensing 1.0 ml per vial into 5 vials. The syringe was rotated 10 more times between each vial. The suspension in the vials was then evaporated in a 50 °C stove for at least 24 hours. A residue of microspheres remained after evaporation which could be weighed and compared between each vial (see figure 3.)

The test was repeated using a microsphere concentration of 15 mg/ml to validate homogeneous dispensing, using a broader range of HoMS concentrations. This suspension was drawn up in two separate 3 ml syringes and dispensed in twelve vials in volumes of 0.5 ml per vial (12x 0.5 ml.). The first 0.5 ml of each syringe served as a flush for the device.

Figure 3 *Holmium microspheres agglomerated in Pluronic in glass vials before evaporation.*

In all vials, microsphere and Pluronic residue after evaporation were weighed. The deviation per vial from the average residue (excluding the flush) was calculated per vial.

Results

The results per vial are presented in figure 4. Results show a variation from the average residue concentration of -10.3% to 5.4% in the 5 ml syringe with a microsphere concentration of 50 mg/ml distributed over 1 ml deposits. A variation of -5.8% to 3.0% was found in the 3 ml syringes with a microsphere concentration of 15 mg/ml distributed over 0.5 ml deposits. The average residue concentration of the 50 mg/ml suspension was 59.9 mg/ml (SD 3.7 mg/ml) and of the 15 mg/ml suspension was 15.9 mg/ml (SD 0.42 mg/ml). The residue concentration is higher than the microsphere concentration since Pluronic also leaves a residue after evaporation. Evaporation of only 0.1% Pluronic resulted in an average residue concentration of 18.6 mg/ml.

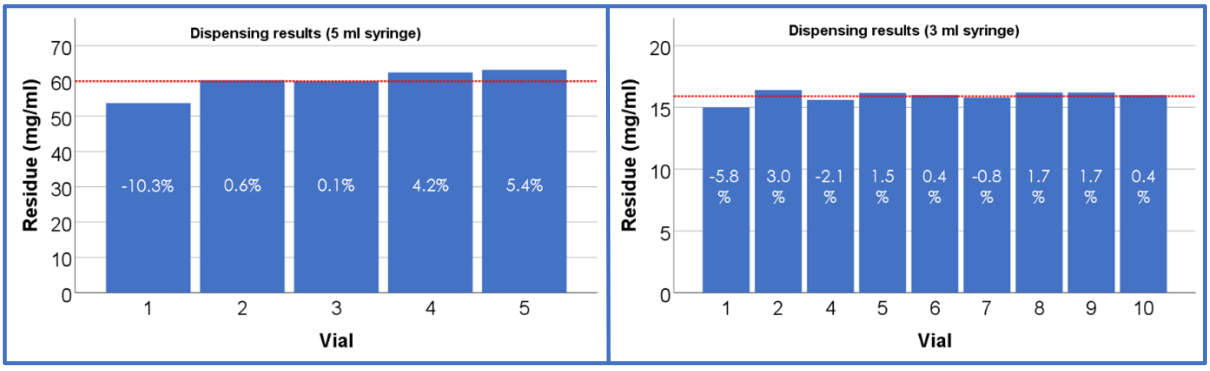

Figure 4 *Dispensing results from the 5 ml (50 mg/ml, 1ml deposits) and 3 ml (15 mg/ml, 0.5 ml deposits) syringes with the injection device for holmium microspheres. Variation from the average residue concentration (red dotted line) ranged from -10.3% to 5.4% and -5.8 to 3.0% from the 5 ml and 3 ml syringes, respectively.*

Discussion

A distribution was provided with a maximum variation of 10.3% by the injection device with both a 3 ml and 5 ml syringe with 15 and 50 mg microspheres per ml suspension. This is far within the previously determined homogenization requirement of 20%. Although sample sizes are too small for statistical analysis, and previous studies show different outcome units (percentage injected ± standard deviation, or Gy), homogeneous suspension seems improved.[6,7]

In both the 3ml and 5 ml syringes, a slight increase in microsphere concentration was seen between the first and the last deposits, even when excluding the flush. This could be explained by a build-up of microspheres against the plunger when it moves towards the tip of the syringe or by the agglomeration of the microspheres in cavities or against protruding edges within the three-way stopcocks. Possibly, increased rotation between each deposit or components with less complex structures could improve homogeneous injection.

Since the rotation and dispensing was performed by hand, factors such as rotation speed, injection speed and injection volume were susceptible to human error. Dispensing errors of just 0.1 ml would result in similar variation as the maximal variation seen now. Since no major outliers are unaccounted for, it seems that these human errors did severely affect the variation during pre-clinical testing.

An exceptional result is that the average residue concentration of the suspension containing 15 mg microspheres per 1 ml 0.1% Pluronic is lower than the residue concentration of 0.1% Pluronic alone, with 15.9 mg/ml versus 18.6 mg/ml, respectively. How this result could be explained is yet unknown. Although the low variation in residue concentration still implies homogeneous injection between deposits, the tests must be reevaluated or repeated to explain this error.

The device achieves variation below 20% for HoMS concentrations in the syringe of 15 and 60 mg/ml. HoMS concentrations used for clinical quantification are often between 10 – 50 mg/ml for CT quantification and between 2.1 – 8.0 mg/ml for MR quantification.[9,10] Validation of homogeneous dispensing by residue measurements becomes less reliable with low concentrations due to measurement errors and scale error. The presented homogeneous dispensing may vary when using lower or higher concentrations. The inter-microsphere interaction becomes less with lower concentrations which may result in a reduced homogenization effect. If the concentration is too high, some microspheres may not become suspended at all, due to increased agglomeration. This research may be repeated if other concentrations are desired.

It is important to notice that this analysis does not show remaining microspheres within the syringes, the stopcocks, or the needle. The injected fraction of microspheres is therefore unknown and will be assessed in the following investigation. When using holmium-166 (radioactive) instead of non-radioactive holmium-165, the radiation can be measured within each component and each deposit using a dose calibrator. Although increased risk of radiation exposure, this would result in more complete microsphere tracking. It could also benefit more accurate analysis of extreme low HoMS concentrations since activity could still be measurable. Another method to achieve a more accurate holmium concentration estimation between deposits is by counting the microspheres using a particle counting and characterization system (e.g., Coulter counter). These systems apply electrical zone sensing, also known as the Coulter principle, to detect, count and characterize micro particles. A similar system is already in use to analyze HoMS morphology during production.[11]

In conclusion it can be stated that an injection device for HoMS was assembled using non-expensive certified components. The injection device showed feasible homogenization and injection of HoMS suspensions.

First application of a new intratumoral injection device for holmium microspheres in a canine patient

Introduction

In collaboration with the Clinic for Companion Animal Health (Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands), a new intratumoral injection device for holmium microspheres (HoMS) was tested for the first time on a canine patient. A client-owned canine was suffering from a solid anal sac carcinoma. An anal sac carcinoma can develop without clinical signs on the inside of the anus. It can only be detected when large enough to protrude outside of the anus or when pain or blood is noticed during defecation. Sometimes the owner may notice increased thirst and urination from the canine which is caused by an increased calcium release of the tumor. Surgical resection is the only curative treatment. However, resection does have an increased risk of damaging the sphincter, blood vessels and nerves, may cause severe infections and permanent incontinence. Metastatic disease often occurs and results in a poor prognosis.[12]

The patient in this case suffered from an anal sac carcinoma of approximately 7 cm in diameter, protruding through the anus and causing significant pain and defecation problems. Due to the size and expected ingrowth through the sphincter and rectum of the patient, surgery was no longer possible. As an experimental treatment, radioactive HoMS were injected under CT-guidance using a novel device for intratumoral injection of HoMS. The aim was to test the device and procedure for feasibility and safety concerning both the patient and the operator. The secondary objective was to investigate the injection fraction (percentage of microspheres administered by the device) of HoMS of the device.

Method

Preparation

Holmium-166 poly(L-lactic acid) microspheres (QuiremSpheres ®), with a mean diameter of 30 µm were delivered on the morning of the procedure. All activity was stored in a single glass vial. In a laminar air flow (LAF) cabinet, the glass vial was measured in a dose calibrator. All preparation procedures were performed using radiation safety measurements by using forceps, gloves, and acrylic and lead shielding. The suspension was diluted with 2.0% Pluronic and homogenized by repeated drawing up and out of a syringe. The required ^{166}Ho activity to reach a planned absorbed tumor dose was calculated using the following equation:

$$D = A \times \frac{15.87}{W}$$

D was the aimed tumor dose in Gy (J/kg), A the required ^{166}Ho activity in MBq and W the mass of the tumor in grams. A tumor density of 1.0 g/cm^3 was assumed. The holmium specific tissue dose conversion factor was 15.87 mJ/MBq.[13] The volume of the suspension to inject was set at 20% of the tumor volume. The activity was evenly divided (± 33.3% of the predetermined activity per syringe) over syringes of 5 ml (see Appendix II). After final measurements, the treatment syringes were capped and stored in a lead transport container.

Injection

The canine patient received sedation and was intubated. The patient was placed stomach-down in a CT scanner (Philips SOMATOM Definition AS) for intermittent CT-guided injections. CTs were made using the following parameters: 120 kV, 400 mAs, 1.0 mm slice thickness, H41s soft tissue filter. The tail of the patient was fixated, and absorbing mats were placed to prevent radioactive contamination. The tumor had the form of an ellipse and was classified in 9 injection-zones. Each zone was divided in sections to indicate planned injections in depth ranging from 2 on the side of the tumor to 4 in the center. This created a dart-board-like pattern to provide distribution guidelines for the operators (see figure 5). To

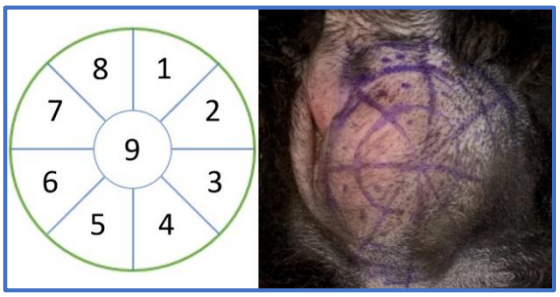

Figure 5 *Injection guidelines on the visual extrusion of an anal sac carcinoma. The guidelines include 9 zones. Within each zone sections were made to represent the number of deposits in the depth, ranging from 2 on the side to 4 in the center.*

establish a sense of the non-visual measurements and location of the tumor, a 75 mm needle was percutaneously placed in the center of the tumor and a pre-injection CT was made.
The 5 ml syringes (see Appendix II) were one by one injected using the injection device previously described, including a 5 mm thick translucent acrylic syringe cover, a 75 mm 22 G (0.718 mm) needle, a 75 mm 20 G (0.908 mm) needle and an empty 5 ml syringe in extension behind the needle which served as a handgrip (see figure 6). All medical component brands and references are presented in Appendix II. Before injecting, the syringe containing the HoMS, was rotated until visual homogeneous with an additional 5 rotations. It was attempted to rotate the syringe between each injection. To achieve a homogeneous distribution within the tumor, the aim was to inject 0.25 ml of suspension per deposit, each deposit 10 mm apart. After injecting each syringe or a series of syringes, the CT was repeated to evaluate microsphere distribution.

Follow-up

After the HoMS injection, a final CT was made. The patient was then transported to the MRI, while under sedation with absorbing mats covering the treatment area. An MRI utilizing both a T1 and T2 weighted sequence with a 2.0 mm isotropic resolution was acquired for visual and quantification purposes. Next a SPECT scan (Philips SKYLight) was made to analyze dose distribution and tumor dose. The SPECT utilized a general-purpose medium-energy collimator, with a Ho166 photon peak of 81 keV (±7.5%), 128 x 128 matrix with 118 KeV ±6% window center. 4.72 mm isotropic resolution reconstruction, and 30 angular positions of 40 seconds per angle by two opposite detector heads. Afterwards the patient was transported to a recovery kennel. Follow-up was planned six weeks after injection.

Results

Preparation

For clarity, all activities were converted to the time at the start of the procedure. The treatment planning aimed at a maximum absorbed tumor dose of 400 Gy, or 3800 MBq evenly distributed over an estimated tumor volume of 150 ml. From the main glass vial, 82% of the activity was divided over 6 syringes of 5 ml (total volume = 30 ml). The remaining 18% was lost in syringes, needles, or vials during preparation. The average activity was 621 MBq per syringe (range: 450 – 821 MBq) with a total activity of 3724 MBq. The specific activity was defined by the microsphere manufacturer at 11.1 MBq/mg ^{166}Ho-PLLA-MS at the time of treatment. The average concentration of holmium-166 microspheres in the syringes was therefore 11.2 mg/ml (range: 3.7-14.8 mg/ml).

Injection

During injections it was noticed that the 75 mm 22G (0.718 mm) needle bent and could hardly penetrate the dense consistency of the tumor. The needle was replaced by a 75 mm 20G (0.908 mm) needle. High density and intratumoral pressure often caused resistance during injection. The needle was then moved a few millimeters until resistance faded. By the haptic feedback of the needle the tumorous tissue was heterogeneous in consistency. Multiple times the resistance faded completely. To prevent injection in blood vessels, it was attempted to withdraw the plunger when no resistance was felt. Figure 6 shows the injection device in use during this procedure.

Figure 6 *Use of the intratumoral injection device for holmium microspheres during treatment of a canine patient with an anal sac carcinoma.*

Flow speed dependent on operator and tissue resistance and was therefore difficult to assess. Injected deposits were often larger than 0.25 ml. This was caused by the large 5 ml syringe in combination with a highly varying intratumoral pressure, and limited view by the acrylic cover. The administration needle was obstructed once and was replaced immediately. After replacement, the needle was not completely secured which caused minor leakage. This was

immediately noticed by the operator, who secured the needle.

After the complete suspension from the first, second, and fourth syringe was injected, CT was repeated to evaluate the microsphere distribution. A lower concentration of microspheres was noticed in the center of the tumor. The fifth syringe was used to compensate for this. The sixth syringe was used to supplement two injection zones which thereafter appeared to have the least microspheres on CT.

From the 3724 MBq in the syringes, 77% (2.84 GBq) was injected by dose calibrator measurements. On average, 13% (range = 3%-28%, SD = 10%) of the activity in each syringe was not administered during injection. From the remaining 10% (= 100% - 77% - 13%), 8% accumulated within the three-way stopcocks and 2% in used materials (gauze, gloves, absorbing mats, etc.). During post-injection evaluation it was seen that the tumor had grown since the previous scan from 150 cm^3 to 175 cm^3. This resulted in a maximum absorbed tumor dose of 258 Gy, when assuming even distribution of 2840 MBq over a volume of 175 cm^3.

Imaging

The holmium was clearly visible on CT as hyperdense spots, and the treatment plan could be adjusted accordingly (see figure 7.B). Since no pre-injection MRI was acquired, no holmium-microsphere quantification could be performed. However, the holmium was clearly visible on MRI up to a point where the holmium artefacts, visualized as black holes surrounded by a white edge, covered most of tumor volume (see figure 7.A). SPECT showed a high dose in the tumor. However, a notable activity of approximately 400 MBq (13.6%) had shunted towards the lungs.

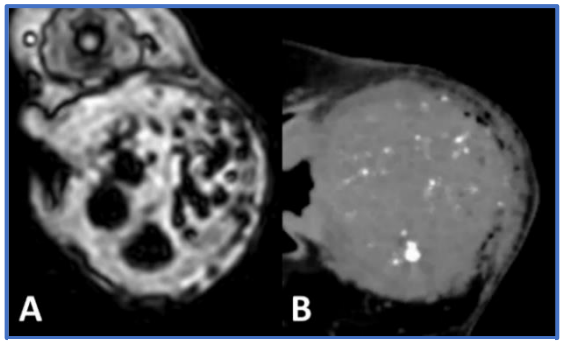

Figure 7 *Post holmium microsphere injection in an anal sack carcinoma of a canine patient. A. MRI shows large holmium artefacts (black holes) inside the tumor. B. Attenuation caused by holmium appears as hyperintense in the tumor on CT.*

Follow-up

The patient recovered from the procedure with minimal side-effects. No complications were noticed from the lung dose or other complications occurred. Six weeks after HoMS injection, the tumor volume was reduced to 125 cm^3 (28.6% volume reduction). A small margin was seen between the tumor and rectum/sphincter and surgical resection was successfully performed. The patient is still alive at the time this was written, 6 months post injection.

Discussion

This study aimed to test an experimental intratumoral injection device, designed to homogenize HoMS suspensions up to the point of injection. This was the first time the device was tested with radioactive HoMS on a live subject.

Device feasibility

Previous studies applying HoMS for intratumoral injection found problematic distribution within the tumor. This was mainly caused by large variations in the HoMS concentration of the injected suspensions.[6,7] The loss of prepared activity was difficult to estimate and systematic loss of 50% was often assumed.[6] This created an estimation of the absorbed tumor dose that was unreliable and could lead to hazardous under- or overdosing. Although this problem was not completely solved by incorporating a new injection device, the dosage has improved. Previous average loss of activity was 40.2% (SD = 17.6%) and was reduced in this case to 23% with an average residue activity in the syringes of 13% (SD = 10%).[6,7]

Procedure feasibility and usability

Although the new injection device was feasible for intratumoral HoMS injection, potential improvements were found. In future injections, the 5 ml syringe will be replaced by a 3 ml syringe. This improves haptic feedback and may reduce the absorbed hand-dose of the user. Large injection volumes are less likely to be needed since human tumors eligible for HoMS injection are often much smaller. A rigid needle is required to precisely inject into the tumor without bending through high tumor density. Here, a 75 mm 20G (0.908 mm) needle was sufficient. Shorter needles, such as 50 mm, probably bent less and could therefore be

feasible at 21G (0.819 mm). Still, no clear causality was seen in this study, or previous studies, between needle diameter and needle blockage.[6,7]

Patient safety

Regarding patient safety, activity shunting to the lungs of almost 14% of the total injected activity was found, possibly caused by injection in blood vessels near the tumor. No complications occurred, however, injection in or near blood vessels needs to be prevented.[14] A arterial or venous phase CT could have indicated large vessels near the tumor and could have been avoided. Another option to prevent injection in blood vessels is periprocedural ultrasound-guided injections.

Operator safety

Regarding operator safety, a hand-dose of 0.48 mSv was found on a ring-dosimeter after injection of half syringe containing 450 MBq. This may be reduced by improved shielding, distance from the source or reduced handling time. It was noticed that the operators held the device near the back of the needle and three-way stopcock to improve positioning of the needle. Since 8% of the total activity accumulated in the three-way stopcocks, this was an additional continuous source of exposure to the hands, next to the main syringes. To prevent this in the future, a larger handgrip is fitted in the extension of the needle to increase distance towards the source. Also, a custom-made acrylic cover was milled to shield the three-way stopcocks for future procedures.

Future improvements

Device performance may still depend on many undiscussed factors such as syringe angle, rotation speed and time, injection time and stationary time. Although rotation by hand is a fast and inexpensive solution, it adds insecurities and transmits vibration to the needle tip. For future improvements, rotation could be automated to maintain a stable and continuous rotation of the syringe. Points of attention when automating the rotation are sterility, durability, dead volume, and usability. Automated injection was not recommended in a clinical setting due to the loss of haptic feedback and control. However, remote injection might create distance and therefore improve radiation safety of the operator. An injection volume limiter could also be implemented.

Conclusion

The intratumoral injection device for HoMS was successfully validated and tested for the first time on a canine patient. The device is feasible, safe, and may be used in future trials. Potential improvements, regarding automatization, could be made in future iterations of the design to reduce human error and further improve operator safety.

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Appendix I. Acceleration of a holmium microsphere

When ignoring drag by surface friction or surface tension the acceleration (m/s^2) of a microsphere can be calculated by the following formula:

$$a = \frac{Fg - Fb}{M}$$

In which ***a*** is the acceleration in m/s^2, $\boldsymbol{F_g}$ is the gravitational force, $\boldsymbol{F_b}$ is the buoyancy force and ***M*** is the mass of the microsphere (1.98E^{-8} g). $\boldsymbol{F_g}$ and $\boldsymbol{F_b}$ can be calculated as follows:

$$Fg = M * g$$

$$Fb = V * \rho * g$$

In which ***g*** is the gravitational acceleration (9.81 m/s^2), ***V*** is the displaced water volume (cm^3) and thus the volume of a microsphere (1.41E^{-8}), and $\boldsymbol{\rho}$ is the fluid density (1.008 g/cm^3).

This results in:

$$a = \frac{(1.98E^{-8} * 9.81) - (1.41E^{-8} * 1.008 * 9.81)}{1.98E^{-8}} = 2.75\ m/s^2$$

Appendix II. Injection device development component overview

Component	Brand	Reference number
Three-way stopcock with rotating male connection	Codan, Steritex	44.5851
Female-female Luer-lock connection	Vygon	892.00
Rotational male-female Luer-lock connection	Qosina	20023
Male-male Luer-lock connection	Vygon	893.00
Syringe (5 ml)	Nipro	SY3-5LC-EC
Syringe (3 ml)	BD Medical	309658
Syringe (1 ml)	Chirana	CH03001LL
Tubing male-female Luer-lock (150 cm)	Vygon	220.150
Anti-backflow valve	B Braun	4094000N
Hypodermic needle (21 G, 50 mm)	BD Medical	301155
Spinal Needle (22 G, 75 mm)	B Braun	4507754
Spinal Needle (20 G, 75 mm)	B Braun	4509575

Appendix III. Overview of requirements and solutions of a holmium microsphere injection device with homogenization by rotation.

Number	Requirement	Solution	Main component(s)
1	Direct applicable device: 3 months prototype 12 months in patient-use	No extended certification or production of components. Only use of CE certified disposables, within their intended use, already commonly used on the department of Nuclear Medicine.	All components with direct contact to the suspension
2a	Control of injection flow: Intuitive flow control down to 0.05 ml/sec	Manual injection by pressing the plunger of a syringe. Control increases with smaller syringes.	Syringes ≤ 5 ml
2b	Control of injection volume: Possible to inject deposits as small as 0.1 ml	Manual injection by pressing the plunger of a syringe. Control increases with smaller syringes.	Syringes < 5 ml
3a	Homogeneous suspension in deposits: Concentration within 20% of the average injected concentration.	Continuous motion of the suspension by rotation in the long axis of a syringe. Visual agglomeration of HoMS happens faster in smaller syringes.	Syringes > 1 ml
3b	No agglomeration in tip or at the plunger of a syringe	Syringe always held in a horizontal position.	Syringe
3c	Horizontal position of the syringe	90-degree angle between the long-axis of the syringe and length of the needle.	Three-way stopcock (figure 2.B)
4	Control of injection location	The free rotation around the long axis of the syringe makes needle placement possible in all angles with minimal limitation of the device	Three-way stopcocks (figure 2)
5a	Minimal change of leakage	Only use of Luer-lock connections. Future iteration: permanent fixation.	All components with direct contact to the suspension
5b	Minimal change of injection of air	Remove air during preparation and no intensive shaking of the suspension is resolved by rotation.	Syringe and rotational three-way stopcock (figure 2.A.)
5c	Minimal loss of control in dead volume	Reduce dead volume to minimal by using components with minimal lumen volume. No unnecessary components and all components measured.	Three-way stopcocks. No male-male or female-female connections, no anti-backflow components.
6a	Minimal change of needle blockage	Hypothetical, increased homogenization also reduces agglomeration in the needle by sudden increases of HoMS concentration. No correlation between needle diameter and blockage at ≥ 20G (0.908 mm)	Needles with diameters ≥ 20G (0.908 mm)

Intratumoral injection of holmium microspheres in *ex-vivo* pancreatic adenocarcinoma

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Intratumoral injection of holmium microspheres in *ex-vivo* pancreatic adenocarcinoma – preliminary results

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Abstract

Introduction: Pancreatic adenocarcinoma holds one of the worst prognosis of all known malignancies. Local treatment options for patients with unresectable pancreatic cancer may increase tumor control and benefit patient survival. This study aimed to test the feasibility of a possible new treatment method utilizing intratumoral holmium microsphere injections.

Method: In this study, non-radioactive holmium-165 poly(L-lactic acid) microspheres (^{165}Ho-PLLA-MS, HoMS) were injected in pancreatic adenocarcinoma in an *ex-vivo* setting. Patients signed informed consent to donate their resected pancreas with tumor to this study after conventional pancreatectomy. HoMS were brought in a suspension of Pluronic and Saline (5 mg HoMS per milliliter suspension) and was homogenized using an intratumoral injection device for HoMS. Pre- and post-injection MRI and CT were analyzed for holmium distribution. Visual holmium artefacts on MRI were manually segmented for a volume estimation.

Results: Three tissue samples of 9.4 ml, 5.6 ml, and 11.2 ml were injected with 4.95 ml, 2.3 ml, and 1.8 ml HoMS suspension, respectively. Pre-injection MRI was used for treatment planning. Two tissue samples were injected using the pre-injection MRI and tumor palpation. In the last-sample additional ultrasound-guidance was performed. Injections were performed with minor leakage or needle obstruction. MRI segmentation showed fractions of the holmium artefacts visual in or near the tumor increased per sample with 26%, 43% and 68%, for sample 1, 2 and 3, respectively. Holmium quantification was performed on sample 3 and gave a simulated mean tumor dose 6 Gy and a mean dose in the largest deposits of 33 Gy. Small deposits were most likely excluded from quantification because of a low resolution. HoMS were not visible on CT. This was most likely caused by automated scanning parameters and a low HoMS concentration.

Conclusion: Preliminary results show that intratumoral injection of HoMS in pancreatic adenocarcinoma is feasible in an *ex-vivo* setting. Improvements regarding microsphere concentration, imaging and quantification may be investigated during the final three patients in this trial. Considering the already reached distribution and injection control, intratumoral holmium microsphere injection may soon benefit patients with unresectable pancreatic cancer.

Introduction

Pancreatic adenocarcinoma is the most common form of pancreatic cancer. Even though pancreatic cancer has the 11th highest incidence of any form of cancer, it also has the third highest mortality.[1] This also represents the poor prognosis of the disease. With a 1-year survival of 20% and a 5-year-survival of 9%, the overall survival of pancreatic cancer remains low.[2] With an increased 5-year survival of 5.9% since 1981, therapy improvement remains minimal.[3] Only 20% of all patients diagnosed with pancreatic cancer are eligible for surgical resection. The remaining patients either have metastasis (50%) or locally advanced pancreatic cancer (LAPC, 30%). Curative treatment for patients with LAPC is only available if they are down staged by intensive neoadjuvant chemotherapy, which is sufficiently effective in 1 out of 5 patients.[4]

Several curative and palliative therapies have been developed and tested over the past decades, with minimal improvement.[3] A treatment that may contribute to primary tumor control is the direct injection of radioactive particles inside the tumor.[5-9] Beta$^-$-emitting (β^-) particles injected inside tumor, in combination with (neo)adjuvant chemotherapy, may increase local tumor control, increase the incidence of tumor down staging and therefore resection. A phase-1/ phase-2 completed trial, successfully injected microparticles incorporating an isotope called Phosphorus-32 (^{32}P) in 50 patients with pancreatic cancer.[5,8] Phosphorus-32 emits β^- particles with an energy of 1.74 MeV and has a half-life of 14.3 days. A local disease control rate of 84% was seen after 16 weeks.[10] This therapy is now available on the European market (Oncosil), however, is not yet implemented in standardized care. Unfortunately, ^{32}P is a non-metal and a pure β-emitter. This makes it difficult to acquire accurate nuclear images and medical imaging for pretreatment planning, periprocedural imaging for treatment confirmation, or follow-up.

Holmium-166 (^{166}Ho) is an isotope that emits β^- particles with a similar energy as ^{32}P (1.85 MeV vs 1.74 MeV) and additionally emits low energy gamma photons (yield 6.7%) with an energy of 80.6 KeV.[11] The gamma photon emission makes it visible on SPECT imaging. Since holmium has a high mass (165,9 u), it causes attenuation of x-rays, causing it to appear hyperintense on CT. And due to the paramagnetic properties of holmium it is also visible on MRI. Holmium is currently being incorporated in poly(L-lactic acid) microspheres (Ho-PLLA-MS, HoMS) and is certified and available in Europe for selective internal radiation therapy (SIRT) of liver malignancies.[12] Since then, quantification of HoMS has been established for both CT and MRI.[13,14] This gives HoMS some clear advantages regarding pre-treatment planning, periprocedural imaging for treatment confirmation, and follow-up when compared with ^{32}P. Furthermore, since holmium has a half-life of just 26 hours, it has a higher dose rate for a total given dose. The activity in a patient would be less than 10% of the original activity, 4 days after therapy. Intratumoral HoMS injection was already performed in veterinary patients and in recurrent head-and-neck squamous cell carcinoma in human patients.[15,16]

This study aims to test the feasibility of intratumoral injection of HoMS in pancreatic adenocarcinoma, in an *ex-vivo* setting. The aim of this study was to develop a new therapy for the treatment of pancreatic cancer with radioactive HoMS.

Method

Patient selection and tissue preparation

Patients diagnosed with resectable primary pancreatic adenocarcinoma were included in this study after signing informed consent. The diagnosis had to be established by pathological and radiological examination and confirmed in a multidisciplinary discussion. The conventional pancreatectomy could be performed by pancreatic tail resection with or without spleen resection, or by Whipple procedure, also known as a pancreaticoduodenectomy. A Whipple procedure includes resection of the duodenum, the head of the pancreas, the gallbladder and bile duct (cholecystectomy), and sometimes the distal part of the stomach. This study was not classified under the Medical Research Involving Human Subjects Act since it does not include patient follow-up, *in-vivo* intervention, or extended patient characteristics.

After conventional surgical resection of the tissue sample, including the tumor, the sample was registered and examined for study approval by a pathologist. Metal clips or staples that could cause MRI and CT intervention were

removed. The tissue remained untreated before HoMS injection. Injection was performed within 2 hours post-resection.

Holmium microsphere preparation

Non-radioactive ^{165}Ho-PLLA-MS of GMP-grade quality were used during this study.[17] The microspheres have a diameter between 15 and 60 micrometer (µm) and a mean diameter of 30 µm (± 5 µm). The estimated holmium content was 19.6% (w/w). Since HoMS quantification by MRI or CT does not require radioactive holmium, and radiation effect in removed tissue is not comparable to that of living tissue, non-radioactive HoMS were used to reduce risk of radioactive exposure.

HoMS were brought in a suspension of 2.0 ml, 0.1% phosphate buffer (Pluronic), diluted with 0.9% NaCl (Saline) to reach a microsphere concentration of 5.0 mg/ml. With use of previous data (see *'Case report: first application of a new holmium microsphere injection device in a canine patient')* it was assumed that approximately 80% of the prepared microspheres reached the tissue during injection (4.0 mg/ml). The suspension was homogenized by repeatedly drawing up and out of a syringe. When homogeneous, the suspension was finally drawn-up in a 3 ml syringe, air bubbles were removed, and the syringe was capped.

Imaging

Treatment planning included tumor volume assessment, nearby vital structures, injection volume, number of injections, deposit volume and number of deposits was based on pre-operative clinical MRI and CT examinations. Tumor volume was estimated by calculating a spherical volume with the largest diameter in each dimension. Study related pre-injection imaging minimally included MRI. Pre-injection MRI consisted of a high-resolution, T2 or T1 weighted spin echo (SPE) and a T2 weighted multi gradient echo (MGE). The treatment plan was re-evaluated with an interventional radiologist using the high-resolution MRI. The purpose of pre- and post-injection MGE was holmium quantification using Qsuite software (Version 2.1). Quantification parameters included a holmium concentration in HoMS of 19.6% (w/w), a specific activity of 12 MBq/mg and a point dose kernel to assess radiation scatter.

Available scanning equipment throughout the study included a 7T MRI (Bruker, ClinScan) with incorporated body-coil, commonly used for small rodents, a CT (MILabs U-SPECT$^+$ /CT) for small rodents, a clinical 3T MRI (Siemens, Skyra) with a knee-coil, a clinical CT (Toshiba, Aquilion Precision) and a clinical ultrasound (US; Toshiba, TUS-X200, Xario 200) with a linear probe. For quantification purposes: the T2 MGE consisted of 11 echo times between 0.8 ms and 20 ms on the 7T MRI and 10 echo times between 1.06 ms and 13.48 ms on the 3T clinical MRI. The CT for rodents had the following parameters: 65 kV, 615 mA, slices 959 x 0.17 mm, pixel spacing 0.17mm, FoV 9.11 x 16.29 cm. The clinical CT used the following parameters: spiral CT, 120 kV, 40 mAs, 80 mA, slices 961 x 0.25 mm, FoV 22.3 cm ⌀. The actual tumor volume was estimated post-injection by segmentation of high-resolution MRI. All used imaging parameters are presented in Appendix I.

Injection

Intratumoral injection was performed using the HoMS injection device described before (see 'Low-cost intratumoral injection device for holmium microspheres'). In addition, the device used 3 ml syringes (BD Medical with Luer-Lok Tip) loaded with the HoMS suspension, a 50 mm 21 G (0.819 mm) hypodermic needle (BD Medical Microlance) and an empty 60 ml syringe as handgrip. The complete system was flushed with 0.5 ml HoMS suspension before injection. Operators were told to rotate the microspheres for 120 seconds and rotate 10 more times just

Figure 1 *Use of the intratumoral injection device for holmium microspheres on ex-vivo pancreatic cancer, sample 1. Syringe shielding, and prototype stopcock shielding were applied to test usability in a clinical setting.*

before injection. If the microspheres visually accumulated between injections, the syringe was rotated again for 120 seconds. The syringe containing the HoMS suspension was kept as horizontal as physically allowed during injections. A low flow speed (<0.5 ml/sec) was advised. The aim was to inject 25% of the clinically assessed tumor volume in the tumor. This could be increased to 33% if pressure allowed it. The flow rate was assessed by video recordings of the injection if possible. Since the microspheres were not radioactive, shielding was unnecessary. Still, acrylic syringe shielding, and 3D printed PLA stopcock shielding was applied to test usability and simulate a clinical setting (see figure 1).

Case series results

Here the results of the intratumoral injections of HoMS in three pancreatic adenocarcinoma tissue samples is described. The injection data is summarized in table 1.

Sample 1

The first sample was retrieved by pancreatic tail resection with additional splenectomy. Pre-operative clinical CT showed a hyperdense lesion with an estimated volume of 14.3 ml. The actual tumor volume was 9.4 ml. Pre-treatment planning was to inject 3.4 ml of suspension by 26 injections over 68 deposits of 0.05 ml, approximately 5 mm apart. The aim was to reach an optimal tumor distribution. Images were acquired using the 7T MRI and the animal CT. The MRI showed a non-vascular tumor with cysts forming inside the tumor and inside the pancreas. Injections were performed by visual guidance and palpation. Low resistance during injection of the suspension was felt, except for one needle obstruction which was easily resolved by retracting the needle a few millimeters. In total 13 injections were made, and 45 deposits injected with an average volume of 0.11 ml. Both horizontal and diagonal injections were placed (see figure 1). This resulted in minimal suspension leakage through intersecting needle tracts.

Post-injection MRI showed clear holmium artefacts in the tumor. On MRI, the high-resolution SPE showed multiple morphological distinctive artefacts (see figure 2). Artefacts formed as almost perfectly spheres with a diameter of up to ±2.5 mm (figure 2.B), small cloud-like artefacts with a diameter up to ±6.5 mm (figure 2.B) and larger cloud-like artefacts with a diameter of up to ±10 mm (figure 2.C). Approximately a quarter of the tumor did not contain any visible artefacts. Pre- and post-injection CT images showed little tissue contrast and HoMS deposits were not seen. A total suspension volume of 4.95 ml was injected. By MRI segmentation, an estimated 1.3 ml (26%) of holmium artefact volumes were found in or near the tumor.

Sample 2

A tumor in the uncinate process of the pancreas was removed by Whipple procedure. The tumor was aligned, and partially grown into the distal part of the duodenum. On clinical CT the volume was estimated at 6.7 ml and redefined by post-injection segmentation at 5.6 ml. Initial planning consisted of just one transduodenal injection with three deposits of 0.4 ml each to improve the deposit recognition and analyze distribution of larger volumes. Only 7T MRI was acquired. High-resolution MRI showed a hyperdense tumor adjacent to the duodenum at one side, and a differentiated border at the pancreatic side. Injections were performed by visual guidance and palpation. A single transduodenal injection was not feasible for a

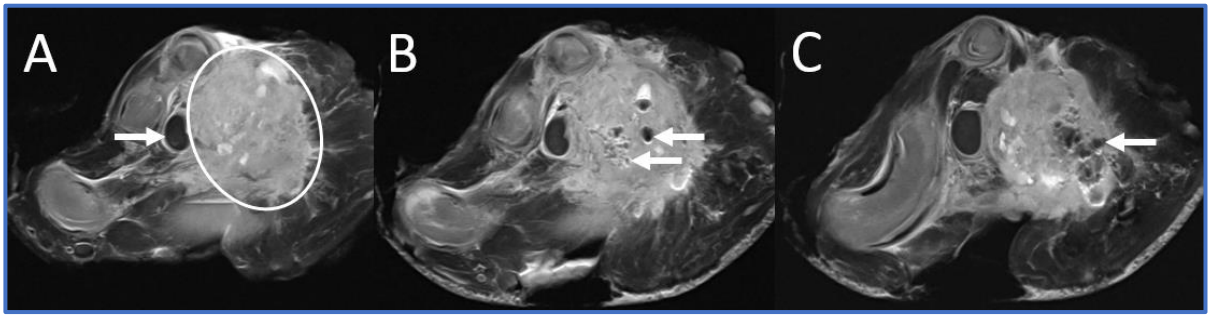

Figure 2 *T2 weighted spin echo MRI of the first tissue sample before and after holmium microsphere (HoMS) injections. A. Pancreatic adenocarcinoma (white circle) before HoMS injection, near the ductus pancreaticus (white arrow). B. Post HoMS injections. Two HoMS artefacts are seen (white arrows). C. Post HoMS injections. A large cloud-like deposit is seen (white arrow).*

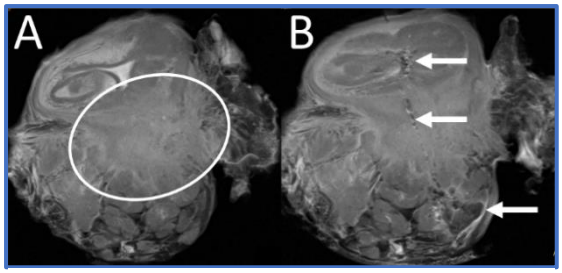

Figure 3 *T2 weighted spin echo MRI of the second tissue sample before (A) and after (B) holmium microsphere injection (HoMS). The tumor is located within the white circle. Artefacts were seen in the duodenum (B. upper arrow), needle tract (B: middle arrow), and just below the tumor (B. lower arrow)*

sufficient microsphere distribution in the tumor. Instead, three transduodenal injections, with one deposit of 0.54 ml each, were made. An average flow rate of ± 0.066 ml/sec was used. During one injection a high resistance was felt. While attempting to inject against this resistance, a sudden drop of pressure caused an overshoot. Also, insufficient rotation caused agglomeration of microspheres in the remaining suspension in the syringe. Therefore, two additional injections of 0.35 ml each were made. All injections were in anterior-posterior direction and no leakage was seen.

Post-injection MRI showed multiple holmium artefacts just behind the tumor in healthy pancreatic tissue. Also, holmium artefacts were found in one injection tracts through the tumor and in the lumen of the duodenum (see figure 3). On the location of the supposed first injection location showed smaller holmium artefacts. The last two deposits formed large cloud-like appearances as seen in figure 3.B. A total suspension volume of 2.3 ml was injected. By MRI segmentation, an estimated 1.0 ml (43%) of holmium artefact volumes were found in or near the tumor.

Sample 3

The last tumor was removed by pancreatic tail resection, including splenectomy. The tumor was in the tip of the pancreatic tail. A tumor volume of 9.6 ml was assumed by clinical imaging. This was later corrected by pre-injection MRI segmentation, showing a clear definable tumor of 11.2 ml. The treatment plan was to inject one large deposit of 0.75 ml in the spherical tail of the tumor and two smaller deposits of 0.45 ml in the remaining gross volume. The goal was to estimate suspension distribution in larger volumes and to test US-guided injection. For this sample, pre-, and post-injection imaging was performed using a clinical 3T MRI. A clinical CT was used for post-injection imaging. All injections were performed using US-guidance. To optimize the US-guided needle placement, first, a flushed needle was diagonally injected into the tumor. When the needle position in the tumor was confirmed, the injection system was attached. Despite the needle flush, air was caught between the connections of the needle and system, causing air bubbles in the tissue. The air was clearly visible in the US, however, the HoMS suspension was difficult to interpret. Still, by US-guidance, it was confirmed that no injections were located outside the tumor. The average flow rate was decreased to ±0.033 ml/sec. One deposit of 0.75 ml and two deposits of 0.45 ml were injected. No needle obstruction, leakage or limiting tumor pressure was found.

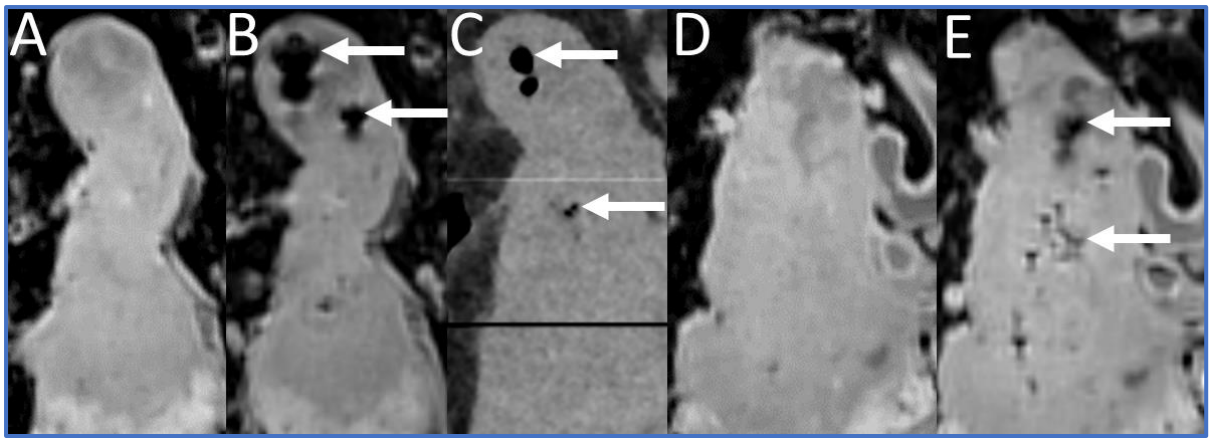

Figure 4 *T1 weighted spin echo MRI RI (A, B, D, E) and CT (C) of the third pancreatic cancer before (A, D) and after (B, C, E) holmium microsphere injection. Artefacts (white arrows) were seen post-injection which were not visible pre-injection (A, D). CT (C) showed the presence of air on the location of some holmium artefacts (B).*

Post-injection MRI showed large artefacts at the tip of the pancreatic cancer where the largest deposit (0.75 ml) was injected (see figure 4.B) and smaller artefacts throughout the rest of the tumor (see figure 4.E). No clear deposits outside the tumor were seen. The tumor could be defined on post-injection clinical CT, however, no clear HoMS deposits appeared. Post-injection CT did confirm the presence of 0.083 ml air in the tumor (figure 4.C), located near the location of the largest artefact on MRI (figure 4.B.) Air and holmium artefacts were indistinguishable. A total suspension volume of 1.8 ml was injected. By MRI segmentation, an estimated 1.3 ml (72%) of holmium and air artefact volumes were found in or near the tumor. After subtraction of 0.083 ml air found on CT, this results in a holmium artefact volumes of 1.22 ml (68%).

Quantification

HoMS quantification was only possible utilizing the clinical MRI of sample 3. The MRI images from the 7T MRI for rodents, used in sample 1 and 2, were incompatible with the Qsuite HoMS quantification software (version 2.1). From the third sample, a multi gradient echo with a resolution of 2x2x4 mm was used (for complete parameters see Appendix I). After quantification, the dose review images were registered with the high resolution T1 weighted spin echo. Qsuite estimated a mean tumor dose of 6 Gy. The holmium and air artefact volumes visible on T1 weighted spin echo MRI (figure 4.B and 4.D) gave a mean dose of 33 Gy (see figure 5).

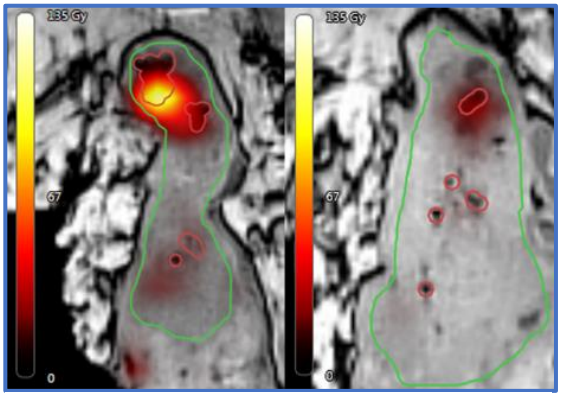

Figure 5 *Dose review images of sample 3 created by Qsuite 2.1 holmium microsphere quantification software. Quantification was performed on the multi gradient echo and results were registered with the T1 weighted spin echo. A mean tumor dose of 6 Gy and a mean artefact dose of 33 Gy was found.*

Discussion

This case series shows the preliminary results of the first injection of HoMS in three human pancreatic adenocarcinomas by a hand-held injection device. Considerable improvements have been made to the injection method and imaging. Microsphere distribution within the tumor was seen in sample 1 and 3 with minimal injection complications. Some injections outside

Table 1 *Summarized injection characteristics per tissue sample*

Injection characteristic		Sample 1	Sample 2	Sample 3
Suspension volume ratio	(Pluronic : Saline)	1:2	1:0.9	1:2.5
Planned tumor volume	ml	14.3	6.7	9.6
Actual tumor volume	ml	9.4	5.6	11.2
Volume injected	ml	4.95	2.3	1.8
Visible suspension in/near tumor	ml	1.3	1.0	1.3
Number of injections		13	5	3
Number of deposits		45	5	3
Volume/deposit	ml	0.11	3x 0.54 2x 0.35	1x 0.75 2x 0.45
Total microspheres injected	mg	19.3	8.9	7.1

the tumor volume were seen which was resolved by US-guided injections in sample 3.

Deposit volume

Deposits between 0.11 ml and 0.75 ml were successfully injected. Larger holmium artefacts were seen after injection of larger deposits, however, the agglomeration of HoMS within the tumor causing a higher concentration may also result in larger artefacts. The morphology of larger artefacts was unpredictable, possibly due to heterogeneous tumorous tissue (figure 2.C). Larger deposits increase the chance of the suspension breaking out of the tumor volume and leaking into healthy tissue. Smaller artefacts are more predictable, however also require more injections thus, increasing the chance of pancreatitis. To effectively treat a tumor's border, which often contains the most proliferating cells, smaller deposits may be the better option. The center of a tumor mass could be treated with larger deposits.

Holmium microsphere distribution

HoMS quantification was only performed on sample 3. Although the multi gradient echo data was available for all samples, some software and scanning limitations occurred. Normally, MRI MGE images could be imported into Qsuite for HoMS quantification. This could estimate the amount of holmium (mg), dose mapping, and dose reconstruction.[14] However, this software was built for SIRT of hepatic tumors and the evaluation of full abdominal images. The 7T MRI, normally used for rodents and applied for sample 1 and 2, acquired images which were not compatible with Qsuite. The clinical 3T MRI could deliver compatible images. However, at the time of acquisition of sample 3, the only sequence with the required echo-times was made for SIRT quantification in hepatic tumors. This resulted in a low resolution of 2 x 2 mm with a slice thickness of 4 mm. Therefore, small deposits with low concentrations were excluded from the quantification, which makes for unreliable quantification. Recommended parameter adjustments are presented in Appendix I. Quantification using CT was also not possible since post-injections scans did not show any increase in HU-values.

The only other method to analyze microsphere distribution was by manual segmentation of clearly visible holmium artefacts. However, this is a sub-optimal method. The small recovery percentage in the first sample (26%) could be explained by small deposits or low concentrations, invisible on MRI. However, if the concentration was too high, artefact volumes may become larger than the actual deposit volume. Furthermore, segmentation of holmium artefacts outside the tumor was not possible since there was little contrast between holmium and air, healthy pancreatic tissue, or connective tissue. Since HoMS concentrations were equal in all samples (5 mg/ml), it could be assumed that more recovery of artefact volumes means a larger fraction HoMS was injected in the tumor. True quantification by relaxation mapping combined with injection of radioactive holmium is the only reliable method to accurately assess the total dose and dose distribution.

US-guided injection

Accurate needle placement using US clearly improved microsphere deposition in the tumor. Post-injection MRI of sample 3 showed that deposits formed near the position where the needle tip was during injection. Although microspheres have successfully been imaged on US in necrotic fluid-filled head-and-neck tumors in previous studies, live imaging of the microspheres was unsuccessful in solid pancreatic tumors.[15] However, air was clearly visible and limited US view. Therefore, injection of air should be prevented in the future.

Microsphere concentration

During this study, the microspheres were brought in a suspension of 2 ml, 0.1% Pluronic, and thereafter diluted with Saline to the correct injection volume and concentration (see table 1). This was chosen since the HoMS are generally delivered by the manufacturer in Pluronic and diluted by Saline for SIRT. However, since the homogenization validation was only performed using 0.1% Pluronic, the Saline might have affected the homogenization performance of the injection device.

In sample 2, severe agglomeration of microspheres in the syringe occurred. This caused fluctuations in microsphere concentration during injections. This may have caused needle blockage, resulting in an overshoot of the holmium microspheres through the needle tract into the duodenum (see figure 3.B).

This study only used a microsphere concentration of 5 mg/ml and assumes 7.1 – 19.3 mg was injected into the tumor. This a relatively low concentration when compared to previous studies (canine: average of 11.2 mg/ml, felines: 200 mg/ per patient, head-and-neck: 200-1250 mg/ml)[16,18] The lower concentration was chosen to prevent too large MRI artefacts. However, this possibly contributed to the microspheres not being visible on CT. Since the specific activity of HoMS depends on the neutron activation time in a nuclear reactor and decay, the HoMS concentration in a suspension can be varied without affecting the total activity. Therefore, the optimal HoMS concentrations need to be investigated for imaging purposes, and the activity adjusted for treatment purposes. The HoMS concentration needs to be higher than 5.0 mg/ml to increase visibility on CT. Optimally, a concentration is found which creates sufficiently small artefacts on MRI, while still being visible on CT.

Imaging

Simultaneously, the MRI and CT parameters may be optimized for holmium quantification. To make the transition between tissue samples and patients smaller, imaging should be performed on clinical scanners. Quantification MRI sequences must include a T2-weighted high-resolution to visualize small deposits and minimal 10 echo times with the shortest echo approaching 1.0 ms. Furthermore, clinical CT parameters were sub-optimal. Because of an automated volume detection on the clinical CT, the electric current/sec (mAs) was reduced to 40 mAs. To measure adequate attenuation caused by holmium, the electric current/sec normally range between 225 mAs and 400 mAs.[13] With these changes to the imaging techniques, it might be possible to quantify HoMS in concentrations of 8.0-10.0 mg/ml. All used and recommended MRI and CT parameters are presented in Appendix I.

Adequate imaging may finally be used for periprocedural real-time imaging of the HoMS injection. MRI-guided injections may be used for real-time dose painting inside the tumor. CT could be used for intermittent dose-painting since real-time CT would result in an increased radiation exposure for both the patient and the operator.

Future perspective

Based on current experience of intratumoral injection of HoMS in pancreatic cancer, a future treatment seems promising and further investigation is recommended. Currently 3 out of 6 tissue samples have been acquired and injected. However, since many factors remain unknown and treatment improvements can be expected, an amendment to increase the sample size might be necessary. It should be noted that this was the first time this treatment method was performed on pancreatic cancer. To accurately simulate and quantify HoMS distribution within the tumor and accurately estimate HoMS remaining in the injection device, radioactive testing seems necessary. Still, through improved imaging and quantification, this treatment may become highly controllable and therefore ideal for pancreatic cancer.

Conclusion

Intratumoral injection of holmium microspheres in pancreatic adenocarcinoma is feasible in an *ex-vivo* setting. Improvements regarding microsphere concentration, imaging and quantification may be investigated during the final three patients in this trial. Considering the already reached distribution and injection control, intratumoral holmium microsphere injection may soon benefit patients with unresectable pancreatic cancer.

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Appendix I: Imaging parameters for holmium microspheres in pancreatic cancer

Table 1 *MRI parameters used and recommended for holmium microsphere visualization and quantification in pancreatic cancer tissue (ex-vivo).*

Tissue sample	1, 2		3		Recommended	
Brand	Bruker, ClinScan		Siemens, Skyra		Siemens, Skyra	
Modality	7T MRI		3T MRI		3T MRI	
Sequence	T2 Spin echo	T2 Multi gradient echo	T1 Spin echo	T2 Multi gradient echo	T2 Spin echo	T2 Multi gradient echo
Tumor visible	+	+	+	+/-	+	+
Holmium visible	+	+	+	+/-	+	+
Quantification	-	-	-	+/-	-	+
Resolution	0.16*0.16	0.26*0.26	0.5*0.5	2.0*2.0	0.5 * 0.5	0.97*0.97
Slice thickness	2	0.26	0.5	4	2	2
Slice spacing	2	2	0,5	4	2	2
Field of view	60*60	49*99	153*224	126*126	48*60	186*186
Repetition time	6200	40	8.95	145	3720	356
Echo number(s)	1	12	1	10	1	10
Echo time(s)	10	0.8 - 20.0	3.69	1.1 - 13.5	54	1.3 – 15.0
Number of averages	1	1	4	3	2	8
Pixel bandwidth	130	1085	260	1530	220	900
Flip angle	180	7	10	33	150	52

Table 2 *CT parameters used and recommended for holmium microsphere visualization and quantification in pancreatic cancer tissue (ex-vivo).*

Tissue sample	3	Recommendation
Brand	Toshiba, Aquilion Precision	Toshiba, Aquilion Precision
Modality	Clinical CT	Clinical CT
Contrast enhanced	-	-
Tumor visible	+	+
Holmium visible	-	+
Quantification	-	+
Resolution	0.22*0.22	0.22*0.22
Slice thickness	0.25	0.25
Reconstruction diameter (mm)	223	223
Kilo voltage peak (kV)	120	100-120
Exposure time (ms)	500	n.a.
Tube current (mA)	80	n.a.
Exposure (mAs)	40	200-400

n.a., not applicable

Future perspective and reflection

Radboudumc university medical center

TU Delft Delft University of Technology

Future perspective and reflection

Future perspective

This thesis focused on the feasibility of injecting a holmium microsphere (HoMS) suspension into pancreatic cancer in an *ex-vivo* setting. By using conventionally resected pancreatic cancer from patients, the injection could be performed without additional risk for the patient. It also creates a controlled environment, which enables the resected tumor to be injected and studied. However, *ex-vivo* investigation is not directly translatable to in-human (*in-vivo*) studies and does not indicate the therapy effect in humans. Furthermore, the final application method, of the intratumoral HoMS injections in *in-vivo* pancreatic cancer is still subject of future research.

This chapter describes three possible application methods to inject HoMS into pancreatic cancer. These methods include ultrasound-guided (US-guided) injection during open surgery, CT-guided percutaneous injection, and endoscopic ultrasound (EUS) injection. Of these therapies, the possible treatment groups, the advantages, and disadvantages are described for future research and application.

Open surgical setting

US-guided open surgery is the fastest applicable injection method since it is closest to the application method in *ex-vivo* pancreatic cancer. A sterile injection device could be implemented with small adjustments. Patients undergoing exploratory surgery to determine surgical resectability, can be injected with HoMS if the tumor is found to be locally advanced. Another patient group that can receive open surgery injection exists of patients that completed chemotherapy and still suffer from locally advanced pancreatic cancer (LAPC), diagnosed by radiological examination. However, the latter group adds additional risk of undergoing open surgery, only for HoMS injection.

The main advantages of injecting HoMS during open surgery, are tumor localization and direct internal control by a surgeon. US-guided injections could be performed with a similar method as the injections in sample 3 of the *ex-vivo* investigation. Also, palpation of the high-density tumor in the soft tissue, and direct haptic feedback in a syringe improves tumor localization and injection control. If complications occur such as needle blockage, leakage, or bleeding it can be detected early and immediately resolved under visual guidance.

The invasiveness of an open surgery is however a paramount disadvantage. Undergoing surgery, including anesthesia, always carries a certain risk. Some patients with high comorbidity are not eligible for surgery and can therefore not receive HoMS injection. If open surgery is the only treatment option for HoMS injection, the treatment's target group would decrease considerably. Using periprocedural imaging for dose distribution evaluation is mostly inhibited during open surgery to reduce the contamination risk of the sterile field. Also, imaging systems incorporated in operation rooms are only available in the larger medical centers. During open surgery, the abdominal cavity may limit the freedom of the injection device and make homogeneous injection more difficult. After injection of radioactive HoMS, the abdominal cavity should be treated as a contaminated work area. Therefore, HoMS injections should be the last possible action before closing the wound. Radiation safety of the surgeon should be closely monitored.

Percutaneous injection

A less invasive application method, yet more technically advanced, is percutaneous injection. Readily available CT can be used for needle placement in the tumor and even for intermittent dose distribution monitoring. Patients can receive HoMS injections after radiological confirmation of LAPC or after recovery of surgical exploratory examination. Patients who may not undergo surgery due to a higher comorbidity can still undergo percutaneous HoMS injection.

CT-guided, percutaneous intratumoral therapies in pancreatic cancer has already been investigated in multiple clinical studies.[1-10] Since percutaneous injection is minimally invasive and HoMS a half-life of 26.83 hours and thus, over 90% of the initial activity is released after 4 days, chemotherapy may start after a relatively short recovery period. The fast recovery contributes to an increased quality of life (QoL), shorter

hospital stays and faster start of adjuvant therapy, such as chemotherapy. Furthermore, the available equipment for CT guided interventions keep enhancing and modern CT-scanners keep improving their image quality, while reducing the radiation dose to the patient.[11]

A disadvantage of percutaneous injections is the approach. To reach multiple tumor locations in the pancreas, perforation risk of the stomach, duodenum, vasculature, or bile ducts increases. Robust needle placement is another challenge. Needles need to be rigid during breathing or organ displacement while injecting HoMS. An accurate needle location is essential for a sufficient dose distribution. If accurate needle placement reduces from a hypothetical 3 mm to 10 mm, this would already have severe effects on the dose distribution, therapy effectiveness and patient safety. However, injection seems feasible since similar studies already successfully performed CT-guided percutaneous injection in pancreatic cancer using needles as small as 25G (0.514 mm).[5] Feasibility and safety of CT-guided HoMS injection in pancreatic cancer should be investigated before initiating larger trials.

Endoscopic ultrasound

The final application method for the injection of HoMS could be endoscopic ultrasound guided (EUS) injections. The purpose of EUS for pancreatic cancer has developed over the past decades from a diagnostic to an interventional purpose.[12] The patient group eligible for EUS is essentially the same as for percutaneous injection. The transluminal approach makes for the least invasive one.

Modern endoscopes produce high quality US images while placed directly next to the pancreas.[13] EUS guidance was already established for micro brachytherapy in pancreatic cancer with phosphor-32 micro particles.[14,15] EUS often equips small needles such as fine aspiration needles (FNA) with diameters no larger than 22G (0.643 mm). The final approach to the tumor may consist of a transgastric or transduodenal injection. Interventions performed by EUS often show less pain after intervention and faster recovery when compared with percutaneous interventions.[16]

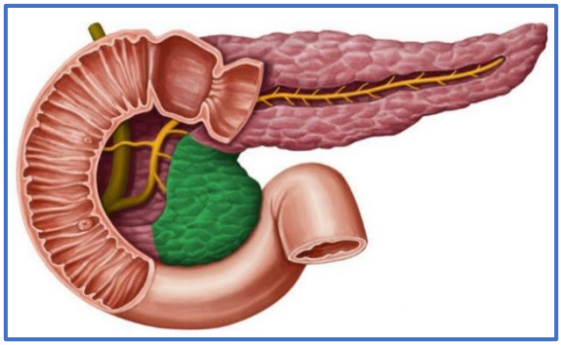

Figure 1 *A schematic overview of the pancreas and duodenum. The uncinate process (green) is difficult to approach by endoscope due to the distal location and angle of the duodenum.*

A clear disadvantage for a technically advanced and high-risk intervention such as HoMS injection, the loss of direct control over the suspension, the needle and the tissue may result in severe complications. If during EUS injection, the radioactive suspension would leak from the tumor into the abdominal cavity or intestines, this is hardly noticeable with EUS. Another limitation of EUS is the freedom of injection approach. The uncinate process of the pancreas is the most difficult to reach using an endoscope, since it can only be reached transduodenal from the distal part of the duodenum, which is positioned in a difficult angle (see figure 1). Another possible limitation is the dead volume. The average dead volume of an endoscope with a needle of 22G (0.718 mm outer diameter, ± 0.413 mm inner diameter) is just below 0.5 ml, however, has a length of over 1 meter. It is yet unknown how microspheres act within this lumen and if microsphere concentrations are predictable during injections.

Future research

Regarding the development of HoMS injection in pancreatic cancer, multiple studies and eventually clinical trials will be necessary to optimize the treatment and prove its effectiveness. In the feasibility study (SLOTH-ex-vivo) promising improvements in the treatment plan have been described. This study will most likely provide the technical feasibility of HoMS injection in pancreatic cancer.

The pilot study (SLOTH-1) will indicate safety and feasibility of HoMS injection in pancreatic cancer in an *in-vivo* setting. Since the sample size in the SLOTH-1 trial will be small, and demonstrating effectiveness is not the aim, this study will most likely be performed in an open-surgical setting which offers optimal control. The research group will include patients eligible to undergo (partial) resection of the pancreas in an open surgery setting as treatment for borderline resectable pancreatic cancer. However, if surgery is aborted due to more advanced disease than initially anticipated, radioactive holmium microspheres will

be injected. Since the patients are already undergoing surgery, this setup involves no additional risks due to open surgery. If the therapy is technically feasible and the results show safe injection, more advanced and less invasive techniques will be investigated. The optimal approach may depend on future development of the microspheres, the suspension, imaging, and available knowledge on the subject.

Reflectie

Van 1 maart 2020 tot en met 27 februari 2021 ben ik bezig geweest met mijn afstudeerstage. Tijdens deze maanden is er veel veranderd, zowel voor mij persoonlijk als voor de wereld om mij heen. In de algemene reflectie blik ik terug op deze maanden en hoe ik mijn afstudeerstage heb ervaren. Ik zal verder ingaan op tijdsbesteding naast het hoofdonderwerp van deze stage, klinische werkzaamheden en ik reflecteer op belangrijke leermomenten uit deze periode.

Algemene reflectie

In maart begon ik met werken op de afdeling Nucleaire Geneeskunde van het Radboudumc in Nijmegen. Ik woonde in Delft en daarom werkte ik maar 2 tot 3 dagen per week in Nijmegen. Naar Nijmegen reizen met de trein kostte veel tijd die ik nuttig besteedde aan het uitvoeren van literatuuronderzoek. Mijn dagen in het ziekenhuis begonnen met oriëntatie en het leren kennen van mijn collega's. Al snel merkte ik een groot verschil tussen het Radboudumc en LUMC in Leiden, waar ik al mijn TM2 stages heb volbracht. In het Radboudumc kende en erkende men het beroep Technisch Geneeskundige vaker. Met weinig moeite kon ik snel connecties leggen met collega's die advies gaven over het behalen van klinische beoordelingen. Eerst ging dit vooral via PhD-studenten en andere studenten van technische geneeskunde uit Enschede. Al snel werden mij kansen geboden bij de interventie radiologie en chirurgie die ik met veel interesse aangreep. Mede door deze ervaring vond ik het minder lastig om, na een periode thuiswerken, in september van Delft naar Nijmegen te verhuizen. In de daaropvolgende periode heb ik veel vooruitgang geboekt in het ziekenhuis. Mijn focus lag voornamelijk op het van de grond krijgen van het onderzoek naar het injecteren van holmium bollen in *ex-vivo* pancreas tumoren (SLOTH ex-vivo). Regelmatig stapte ik de kliniek in om klinische ervaring op te doen. De vrijheid van meelopen of assisteren waar en wanneer het maar uitkwam liep snel af, en er moest, vanwege de COVID-19 pandemie, met meer voorzichtigheid en vooral meer afstand worden gekeken naar klinische blootstelling.

Dit moment van mijn afstudeerstage gaf mij een dubbelzijdig gevoel. Enerzijds had ik een voldoende zelfstandige houding ontwikkeld binnen het Radboudumc om zonder veel direct contact wel resultaat te behalen, anderzijds voelde het weleens eenzaam op de werkvloer. Deze tijd ging samen met meer onzekerheid, en minder sociale interactie maakte dit tot een uitdaging. Gelukkig kon ik daar met een aantal contactpersonen en een flexibele instelling goed doorheen komen en ben ik de inzet voor mijn studie en werk niet kwijtgeraakt. Ondertussen had ik toch meer mensen leren kennen binnen en buiten de afdeling. Ook al was het niet altijd zichtbaar of mogelijk om te uiten, ik vond de werksfeer op de afdeling voornamelijk positief, ondersteunend en gezellig. Daarom kijk ik uit naar een nieuwe periode, waarin alles voelt als vanouds maar niks meer zal zijn zoals vroeger.

Tijdsbesteding

Naast het uitvoeren van het onderzoek dat staat beschreven in deze thesis, heb ik veel andere ervaringen opgedaan binnen en buiten de muren van het Radboudumc. Via het bedrijf Quirem Medical ben ik in contact gekomen met mijn stagebegeleider, Frank Nijsen. Quirem is de fabrikant van de holmium microsferen en is gedreven door innovatie en onderzoek. Daarom ben ik vaak met de medewerkers van Quirem Medical in contact geweest over de mogelijkheden van de therapie, het opzetten van vervolgonderzoek en de samenwerking tussen Quirem en het Radboudumc. Hierbij heb ik mij kunnen presenteren als een medisch-technologisch adviseur over pancreaskanker vanuit een medisch centrum naar een fabrikant van een medisch hulpmiddel. Omdat ik voorheen juist was betrokken bij medisch wetenschappelijk onderzoek vanuit het oogpunt van de fabrikant, was dit een unieke ervaring die ik erg interessant en leerzaam vond.

Aan het begin van deze stage kon ik mij verdiepen in medisch wetenschappelijk onderzoek dat al van start was gegaan binnen de afdeling. Hierbij heb ik veel geleerd over de logistiek, communicatie en

voorbereiding, wat voorafgaat aan onderzoek doen. Het lezen van dossiers, reserveren van apparatuur, communiceren met collega's, het bereiken van patiënten of het afnemen van informed consent is slechts een fractie van het voorbereiden en uitvoeren van klinisch onderzoek. Deze ervaringen hebben mij geholpen bij het opzetten en uitvoeren van het pancreas onderzoek (SLOTH ex-vivo).

Ook heb ik een onderzoek aanvraag geschreven en ingeleverd bij de medisch ethische toetsingscommissie. Een opzet was al gemaakt maar het geheel moest worden herschreven en aangevuld. Dit betreft de pilotstudie, en het vervolgonderzoek van mijn thesis, holmium microsfeer injectie bij patiënten met pancreaskanker (SLOTH-1). Dit vereiste veel communicatie met medisch professionals, onderzoekers en de fabrikant. Vanwege de multidisciplinaire insteek van dit onderwerp, heb ik het genoegen gehad om in gesprek te gaan over de studie-opzet met chirurgen, interventie radiologen, nucleair geneeskundigen, MDL-artsen, klinisch fysici, onderzoek adviseurs, fabrikanten en natuurlijk technisch geneeskundigen. De grootste aandachtspunten van deze aanvraag waren het investigational medical device dossier (IMDD), een failure mode and effects (FMEA) analyse en het onderzoeksprotocol.

Wekelijks waren er research-meetings van zowel de afdeling nucleaire geneeskunde (NucMed-meeting) als de minimally invasive image-guided intervention center (MAGIC). Hierin werden onderwerpen gepresenteerd en bediscussieerd van nucleaire biomarkers in celkweken, tot de nieuwste imaging methodes van een welbekend virus dat in omloop is. Zelf heb ik hier meermaals mijn studie-opzet, resultaten en thesis mogen presenteren. Daarnaast werden er in de laatste 1-2 maanden dosimetrie meetings georganiseerd om meer te leren over micro en macro dosimetrie in mensen, dieren en cellen.

Klinische ervaring

Tijdens mijn stage heb ik een deel van mijn tijd besteed in de kliniek. Persoonlijke doelen waren daarbij het ontwikkelen van medische vaardigheden, persoonlijke ontwikkeling, ervaring opdoen met het uitvoeren van medisch-wetenschappelijk onderzoek en het verdiepen in de zorg rondom pancreaskanker.

Vanaf september heb ik wekelijks het pancreas oncologie (PACON) multidisciplinair overleg gevolgd. Tot en met kerst heb ik slechts 2 keer het PACON-overleg gemist. Hier heb ik het grootste deel van mijn kennis over pancreaskanker en de zorg eromheen geleerd. Alle aspecten zoals monitoring, stadiëring, ziektebeloop, interventies, onderzoeken, klinisch redeneren, palliatieve en curatieve zorg, follow-up en de wens van de patiënten werden keer op keer besproken. Streekziekenhuizen uit de buurt tot zover als ziekenhuizen in Groningen sturen patiëntendossiers door naar dit overleg voor advies. Naast het opdoen van medische kennis over pancreaskanker, nam ik ook deel aan deze meeting om patiënten te includeren voor het onderzoek. Deze meeting werd dan ook vaak opgevolgd met het uitgebreid lezen en analyseren van patiëntendossiers voor mogelijke inclusie in het onderzoek. Ik heb het gevoel basiskennis te hebben ontwikkeld over pancreaskanker die mij kan helpen bij het uitvoeren van toekomstig onderzoek. Daarbij is het belangrijk om professionals te betrekken bij sleutelmomenten om zo een goede inschatting te kunnen maken en gedachten uit te kunnen spreken. Meermaals heb ik van artsen en chirurgen te horen gekregen dat ik er goed bovenop zit, en duidelijk aangaf dat een patiënt in aanmerking kwam voor de SLOTH ex-vivo studie. Patiënten moesten vaak opnieuw worden besproken door de onderzoekers zodat er een verdeling kon worden gemaakt tussen de lopende studies van verschillende afdelingen.

Daarnaast heb ik veel eenmalige klinische ervaringen opgedaan, zoals het bijwonen van een CT-gestuurde punctie inclusief periprocedurele beeldverwerking, een open-buik Whipple-procedure, het aan de lopende band infusen leggen voor contrast-enhanced CT's, assisteren bij klinisch onderzoek en zelfs meelopen bij Diergeneeskunde in Utrecht. Ook heb ik erg veel technisch geneeskundige (TG'er) ontmoet, van wie ik veel heb geleerd. Het was uitzonderlijk hoe elke TG'er zijn eigen onderwerp en eigen vaardigheden-set had ontwikkeld. Het hebben van basiskennis van technologie is een start, maar het werken met en het kunnen toepassen is het iets anders. Daarvoor is oefening en toewijding nodig, en ik vond het een bijzondere ervaring om dit van elke TG'er te mogen meemaken. Zelf hoop ook door veel oefenen een set vaardigheden te ontwikkelen die ik kan toepassen in mijn toekomstige loopbaan, dan wel bij het uitvoeren van medisch wetenschappelijk onderzoek.

Bij het meelopen met medisch wetenschappelijk onderzoek heb ik voor het eerst een informatiegesprek meegemaakt met een terminaal zieke patiënt. Ik had mijzelf voorgenomen hiervan te leren voordat ik zelf patiënten zou gaan informeren en includeren voor onderzoek. Niet veel later had ik mijn eerste gesprek met een patiënt die stond gepland voor chirurgische resectie van de pancreas vanwege pancreaskanker. Uiteindelijk heb ik 6 verschillende patiënten mogen ontmoeten, en er 5 kunnen includeren.

Leermomenten

Over de afgelopen 11 maanden heb ik veel geleerd. Ik hoop dat ik met deze ervaringen verder ben ontwikkeld richting een volwaardig technisch geneeskundige. Ik benoem 'richting een volwaardig technisch geneeskundige', omdat ik van mening ben dat je altijd kan bijleren en ontwikkelen. Voor het schrijven van dit onderdeel heb ik teruggeblikt in de korte klinische beoordelingen (KKB's) van deze stage. Terugkerende omschrijvingen daarin zijn *inzicht, toewijding en interesse*. Ik ben blij dat deze punten worden benoemd aangezien dit onbewust het plezier is dat je in je werk of studie stopt. Ik beschreef eerder de hoeveelheid obstakels die men tegenkomt bij het uitvoeren van medisch wetenschappelijk onderzoek. Ook hierbij zijn toewijding en interesse nodig om dit te zien als een uitdaging en het met beide handen aan te pakken. Als ik hier anders had ingestaan, verwacht ik niet hetzelfde resultaat te hebben behaald.

Ook *zelfstandig* en *proactief* worden vaker genoemd, en dit is zowel een sterk punt als een verbeterpunt. Tijdens deze stage heb ik geleerd dat ik niet alles alleen moet doen. Soms probeer ik het wiel opnieuw uit te vinden, of communiceer ik niet met mijn omgeving. Ik ben niet bang om toe te geven dan ik nog veel kan leren van anderen, maar ik moet dit vaker gaan tonen. Op de afdeling en in het ziekenhuis zijn zoveel mensen die al jaren onderzoek doen, zorg leveren of innoveren, en die mij sneller op weg kunnen helpen dan ik kan in mijn eentje. Daarnaast is overleg essentieel voor vooruitgang en leidt gevraagd en ongevraagd advies vaak tot een betere uitkomst. Onderzoek doe je niet alleen voor jezelf, maar juist om met anderen te delen, te overleggen en te discussiëren.

Het was een bijzonder leermoment om met patiënten te spreken met een vooruitzicht zo somber als dat van pancreaskanker. De moed en volharding van deze patiënten vond ik inspirerend. Bij de eerste patiënten focuste ik veel op wat ik wel en niet kon zeggen en minder op de patiënt zelf. Gelukkig leerde ik snel en focuste ik mijn aandacht meer op de patiënt en hield ik mijn onderzoek in mijn achterhoofd. Ik begon gesprekken vaak met iets simpels of iets herkenbaars om te peilen hoe de patiënt zich voelde of wat voor stemming er was. Na het uitleggen van mijn onderzoek begon ik expliciet te vragen naar onduidelijkheden of zorgen van de patiënt. Hierdoor kwamen er vaker punten naar boven die extra uitleg vereisten. Onzekerheid leek altijd de grootste zorg en veroorzaakte chaos. Daarom wilde ik graag duidelijkheid en rust bieden en verdere zorgen ontnemen. Dit deed ik naar mijn mening altijd binnen mijn verantwoordelijkheden en kennis. Vanwege de heftige situatie van de patiënten heb ik deze gesprekken na de eerste patiënt alleen uitgevoerd en daar mijn tijd voor genomen. Achteraf voerde ik vaak een zelfreflectie uit a.d.h.v. een KKB.

Dit is slechts een deel van de talloze leermomenten van deze stage. Ik heb in deze periode 9 KKB's, 1 OSAT en 1 TMPA beoordeling verzameld, 4 KKB's zelf geschreven en een logboek bijgehouden vanaf half oktober met daarin 43 medisch gerelateerde ervaringen. Ter afsluiting; ik vond het een waardevolle en leerzame periode.

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Annex A: Intratumoral therapies for locally advanced pancreatic cancer – a systematic review

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Abstract

Introduction: Pancreatic cancer holds one of the worst prognosis of all malignancies. Patients suffering from locally advanced pancreatic cancer (LAPC) have a minimal chance of receiving curative surgery. Intratumoral therapies have been studied as novel treatment options for improved local tumor control. This systematic review aims to evaluate existing intratumoral therapies and provides an overview of the procedural safety and survival in patients with unresectable locally advanced pancreatic cancer.
Method: A literature search was conducted in PubMed, EMBASE and Cochrane Library for English written articles up to March 23rd, 2020. All study designs involving at least 5 patients with LAPC who were treated with at least one intratumoral therapy were included. Primary outcomes included safety, and survival. All included studies were critically appraised by the Newcastle-Ottawa Scale. Survival between multiple modalities was statistically analyzed using a one-way ANOVA.
Results: After evaluation of the 1404 articles yielded by the systematic search, 55 studies containing 1993 patients were included. Included treatment modalities consist of iodine-125 (I125) brachytherapy (27 studies), phosphorus-32 (P32) brachytherapy (5), immunotherapy (11), intratumoral combination therapy (5), intratumoral chemotherapy (4), palladium-103 brachytherapy (2), iridium-192 brachytherapy (1) and Mistletoe (Viscum album L) injection (1). Survival ranged between 5.5-15.0, 5.2-16.0, 5.8-13.8, 11.0-23.0, 9.0-16.2 months for I125, P32, immunotherapy, combination therapy and intratumoral chemotherapy, respectively. A statistically significant difference (p = .026) was found between the survival of combination therapies when compared with the I125 brachytherapy, P32 brachytherapy and intratumoral immunotherapy. Chances of severe complications (≥ 3 by Clavien-Dindo Classification) of 6%, 198%, 77%, 19% and 0% were reported for I125 brachytherapy, P32 brachytherapy, immunotherapy, combination therapy and intratumoral chemotherapy, respectively.
Conclusion: A wide variety of intratumoral therapies is described and an overview is reported. Although evidence is limited to case series and cohort studies most intratumoral therapies seem feasible and safe as treatment for patients with locally advanced pancreatic cancer. Combined intratumoral therapies may have the best survival benefit for patients who are ineligible for resection when compared with single intratumoral therapy.

Introduction

Pancreatic cancer is diagnosed in over 440.000 people worldwide every year and incidence increased by 55% over the last 25 years. The mortality is similar to the incidence due to the poor prognosis of this malignancie.[1] Since 2014 an improvement in 5-year survival was seen of 3%. Still, with a 1-year overall survival of only 20% and 5-year survival of 9%, pancreatic cancer is one of the most aggressive forms of cancer.[2] Resection can be performed in just 20% of all cases and is the only curative treatment option. For the remaining 80%, local advancement or distant metastasis (stage IV) make resection obsolete.[3] Around half of all patients with pancreatic cancer suffer from distant metastasis at the time of diagnosis which makes resection futile.[4] The remaining 30% suffer from locally advanced pancreatic cancer (LAPC). The most commonly used criteria for LAPC are those from the National Comprehensive Cancer Network (NCCN) guidelines, defining LAPC as >180 degrees arterial involvement or unreconstructable venous involvement without evidence of distant metastasis.[5] Often, tumor involvement in the superior mesenteric artery, celiac axis or common hepatic artery or definite occlusion of the superior mesenteric vein or portal vein make pancreatic cancer unresectable.[6]

The current therapy of choice for LAPC is palliative chemotherapy with FOLFIRINOX (a combination of 5-Fluorouracil, irinotecan, leucovorin and oxaliplatin) or nab-paclitaxel, with response evaluation after 4 and 8 cycles. If metastases remain absent and tumor shrinkage or downstaging is observed, resection may still be performed in around 20% of these cases.[7] Gemcitabine was the recommended palliative therapy for over a decade and is still used in patients with a WHO performance score of 2 and higher.[8] Recent studies on the outcome of FOLFIRINOX-based treatment in patients with LAPC found an overall survival of 14.8-24.2 months, however the majority of these patients also underwent radiotherapy or resection.[9, 10] For patients with severe comorbidities these extensive combination treatments are often considered impossible.[11] Some studies even claim almost half of the elderly patients (>65 yr.) with not metastasized pancreatic cancer do not undergo either chemotherapy or surgery, possibly due to disease progression or developed comorbidities.[12] For these under-treated patients and for patients with metastasis (stage IV), survival decreases sharply with a 5-year survival of only 3% for stage IV pancreatic cancer.[13]

For patients with stable unresectable disease after 2 months chemotherapy, local ablation is sometimes applied in clinical trials in hope of controlling local progression and prolonging survival.[14, 15] Local ablation techniques used for LAPC include: radio-frequency ablation (RFA), irreversible electroporation (IRE), stereotactic body radiation therapy (SBRT) and high-intensity focused ultrasound (HIFU). Although ablation is considered feasible, it is also associated with substantial morbidity and mortality.[14] Effectiveness of additional local ablation is disputable due to the lack of comparison studies. Overall, small non-comparing case studies, often containing selection bias, result in a widely varied survival from 5.0 up to 25.6 months.[14] The conventional treatment planning for patients with LAPC are presented in figure 1.[8]

Ablation therapies are often limited by intra-operational control. Although image processing is widely studied to enhance the direct visual feedback of ablation, the ablation accuracy is still limited. The so-called ablation zone, including the gross tumor volume, is surrounded by a security edge. This security edge needs to be present to protect vulnerable tissue and vessels from thermal damage and protein denaturation.[16] However, tumor surface heterogeneities which branch into the security edge often cause disease recurrence when not treated accordingly. This is especially important with LAPC where tumors often encase local vessels which prevents sufficient thermal ablation or increases complications like internal hemorrhages.[17] A study by Rombouts et al. (2014) evaluated the clinical outcomes of multiple ablation techniques in LAPC.[14] Rombouts et al. (2014) reported a

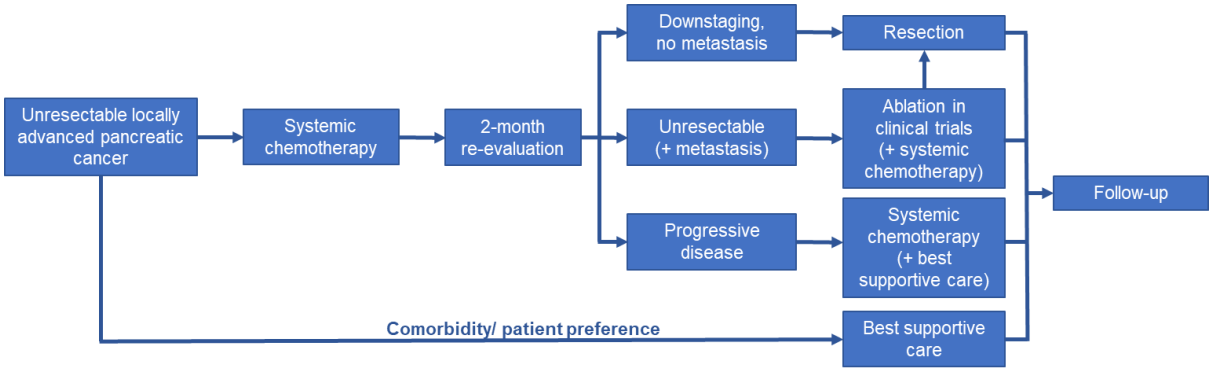

Figure 1 *Flow chart of treatment options for unresectable locally advanced pancreatic cancer (LAPC). First choice chemotherapy consists of FOLFIRINOX or nab-paclitaxel, depending on patient performance status. Treatment planning may vary depending on country and study participation.*[15]

maximum median survivals of 12.6, 20.2, 24.0 and 25.6 months for HIFU, IRE, SBRT and RFA, respectively.

A new therapy fow LAPC is required. Optimally without heat-sink limitation, precise delivery of the treatment and improved therapy prediction. Over the past decades, novel intratumoral therapies and approaches for pancreatic cancer have been studied and innovated worldwide. Advancements of immunological pathways, advanced image processing, therapy control and personalized treatment planning have changed the approach to achieve local tumor control. With less invasive application methods like angiographically or endoscopic ultrasound (EUS) complication rates may decrease as well as hospital stays and healthcare costs. Intratumoral therapies may be better controlled with medical imaging and adjusted for optimal tumor treatment.

This systematic review aims to evaluate existing methodological approaches for intratumoral treatment of unresectable LAPC and provides an overview of their procedural safety and impact on survival.

Method

This systematic review was conducted and reported conform to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[18] The primary method and inclusion/exclusion criteria of this review were specified in advance in consultation with a Biomedical Information Specialist and the following authors (Y.W.; N.K.; F.N.). It was thereafter registered on PROSPERO, the international prospective register of systematic reviews (registration ID: CRD42020212862).

Search strategy

A literature search was conducted in PubMed, EMBASE and Cochrane Library for English written articles from all precedents up to March 23rd, 2020. One preliminary abstract was updated in July 2020.[19] The literature search was performed using medical domains combined by 'AND' between domains and within the domain by 'OR'. The first domain contained terms regarding pancreatic cancer, the second regarding intratumoral therapy and the third regarding unresectable LAPC. Search terms were restricted to MeSH, title, abstract and keywords. The complete search strategy for each library is presented in Appendix A*.

Definitions

LAPC was defined by LAPC as stated in the NCCN guidelines or AJCC guidelines that was not, at any time during the study or follow-up, resected.[6, 15]

Intratumoral therapy was defined by a therapy with intention to treat or control the primary pancreatic cancer with the goal to be delivered and functional only within the gross tumor volume. Infusion therapy, stenting, ablation, or post-resection treatments were not defined as intratumoral therapy.

Study selection

After a first scan to remove duplicate publications and reviews, the titles and abstracts were scanned for inclusion and exclusion criteria. There was no limitation to

study design or publication date. Articles had to be published in a registered journal defined by the SCImago Journal & Country Rank.[20] Publications limited to an abstract were not excluded if the information was adequate, as described below. If multiple studies contained the same research group, only the latest published article was admitted. If there was uncertainty regarding inclusion, a second author was invoked (N.K.).

Studies containing five or more human patients with LAPC, treated with at least one intratumoral therapy were included. Dose-escalation studies needed at least ten patients with a similar dose or fraction to be included due to the large variety in effects, complications, and survival between doses. If a study contained a majority of LAPC with additional patients diagnosed with resectable pancreatic cancer or distant metastasis, the study was included and results were separately noted, if possible.

Quality assessment

All studies passing the full text assessment were critically appraised according to the Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies. The NOS is a validated scoring system with appraisals for case-control studies and cohort studies. A total of 4 studies using randomization were included in the cohort evaluation. A total of 9 stars (☆) could be appraised per classification, 4 by selection, 2 by comparability and the last 3 by either exposure or outcome of interest for case-control and cohort studies, respectively. The complete scoring criteria are presented in Appendix B*. Studies with 5 stars or more were considered of good quality. Studies with less than 5 stars were not excluded.

Data selection

Data on cancer stage, metastasis, (neo)-adjuvant therapy, complications by the Clavien-Dindo Classification[21], median survival, local tumor control or response rate by WHO and/or RECIST criteria (response evaluation criteria in solid tumors) were extracted when available.[22] Furthermore, study characteristics such as design, country, population characteristics and sample size were extracted from the included studies.

Statistical analysis

Most outcomes were descriptive and due to the heterogeneity of the included studies, no meta-analysis was performed. Median survival between two groups was statistically analyzed using an independent sample t-test and between multiple groups with a one-way ANOVA without assuming equal variances (Welch analysis of variance). For statistical analysis and data analysis, IBM SPSS Version 25 (IBM Corporation, Somers, NY, USA) and Microsoft Excel for Microsoft 365 was used. Statistical significance was defined by a two-tailed p-value of < 0.05.

Results

Starting with 1404 publications, after title and abstract screening for duplicates and exclusion criteria, 1323 studies were excluded. A detailed selection flow chart is shown in figure 2. Just 81 studies entered full text assessment. Of these 81 studies, 26 studies were excluded because of a too small sample size (12), not reporting relevant outcomes (10) or intervention not meeting the inclusion criteria (4). Finally, 55 clinical studies with a total of 1993 patients were included for quality assessment (figure 2). The results from the quality assessment are reported in Appendix C*.

The included studies comprise 6 different intratumoral treatment modalities: iodine-125 (I125) brachytherapy (27 studies), phosphorus-32 (P32) brachytherapy (5 studies), immunotherapy (11 studies), combination therapy (5 studies), chemo injection therapy (4 studies), and individual therapies (4 studies). One study contained both I125 therapy and combination therapy. Many of the included studies had the following inclusion criteria in common: age ≥ 18, life expectancy of more than 3 months, and an adequate hepatic, hematologic, immune, and renal function. Gemcitabine was the most used form of chemotherapy. Only one study combined intratumoral therapy with FOLFIRINOX chemotherapy.[19] All surgery that did not directly affect the pancreatic cancer was ignored in this analysis. This includes bile ductjejunostomy, cholangio-

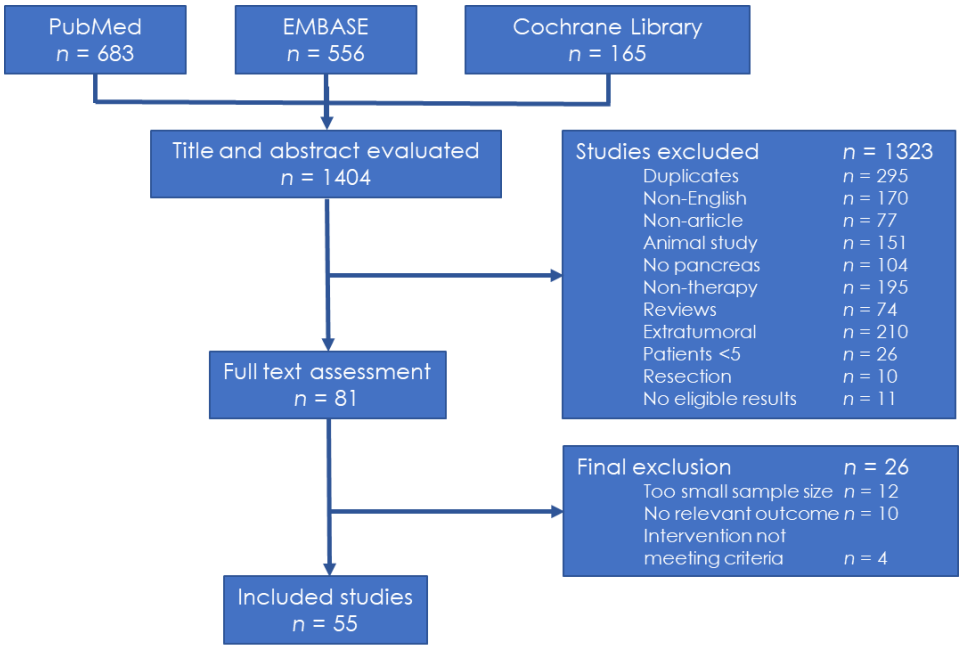

Figure 2 *Study selection flow chart.*

jejunostomy, gastrojejunostomy, biliary/gastric bypass, and stent placement.

For clarity, the results are reported per modality. The treatment modality, complications and survival are described. Extended treatment methods regarding intratumoral delivery of a therapy was underexposed in most articles.

Iodine-125

I125 is commonly used in nuclear medicine for radiation therapy such as brachytherapy. I125 decays by electron capture and emits characteristic photons and electrons with a maximum gamma energy of 35 KeV and a tissue penetration of 17 mm.[23] With a half-life of 59.4 days, I125 has a relatively lower dose rate compared to other brachytherapy isotopes. However, no clinical evidence is available claiming that tumor damage is affected by the dose rate at a given total dose. The seed's length ranges from 4.4 to 4.6 mm with a diameter of less than half a mm.[24] Included studies implanted 10 to 150 seeds in one or multiple iterative operations depending on tumor volume, characteristics and response.

Of the 27 studies applying Iodine-125 brachytherapy on 1095 patients suffering from LAPC, 10 studies had a retrospective study design[25-34], 16 an open-label prospective design[35-50] and one compared I125 combined with Gemcitabine and S-1 chemotherapy, versus Gemcitabine and S-1 chemotherapy alone in a randomized controlled trial (RCT).[51] For the application method, 14 studies implanted the seeds intra-operatively (IO) in an open approach using of X-ray, CT, or US guidance. One study from 1990 describes how the needle location was determined by palpation and feel, by placing one hand behind the head and body of the pancreas.[25] Eight studies used percutaneous implantation guided by US or CT. Since 2006, five studies implemented EUS to deliver the radioactive seeds to the tumor transgastric or transduodenal. The minimal peripheral dose (MPD) ranged from 51.5 to 167 Gy with an average of 127 Gy. Out of 612 patients in 16 studies, a total of 37 (6.0%) ≥grade 3 complications by Clavien-Dindo Classification occurred. The most common complications reported were gastrointestinal hemorrhages, pancreatic fistula, leukocytopenia and different intra-abdominal infections like pancreatitis and cholangitis.

The median overall survival ranged from 5.5 to 16 months. Liu, K et al. (2014) found the highest median survival of 16 months in 30 patients.[28] However, the included patients in the study by Liu, K et al. (2014) were on average 38 years old, while the average age of the patients in the remaining studies was 62 years. Complete response was only reported in three cases by Zhongmin et al. (2010).[50] Eventually, all patients included by

Zhongmin et al. (2010) died as a result of primary tumor progression or distant metastasis.[50] The overall survival of 217 patients who received I125 implantation, without chemotherapy ranged from 5.5 to 14 months with an average of 7.9 months. The overall survival of the 656 patients that did receive chemotherapy adjuvant to I125 implantation ranged from 7 to 15 months with an average of 10.1 months. Although average survival seems to improve by chemotherapy, no statistically significant difference was seen between the survival of patients undergoing I125 implantation with or without systemic chemotherapy (p = 0.06). In the RCT a statistically significant difference ($p < .05$) was established between the survival of I125 combined with chemotherapy (11.84 months), versus chemotherapy alone (10.40 months).[51] An overview of the study results of I125 brachytherapy are shown in table 1.

Table 1 Results of Iodine-125 brachytherapy for locally advanced pancreatic cancer (continued on next page)

Reference	No. of patients	Metastasis *n* (%)	Application method	(Neo)-adjuvant therapy	≥Grade 3 complications *n* (%)	Median survival (months)	NOS ☆
Dobelbower et al.[35]	12	2 (17)	IO, X-ray	Chemoradiotherapy	1 (8)	15	8
Du et al.[36]	100	40 (40)	EUS	Chemotherapy	0 (0)	7	8
Goertz et al.[25]	11	2 (18)	IO	Radiotherapy	1 (9)	8	7
Jin et al.[37]	22	8 (36)	EUS	Chemotherapy	n.r.	9	6
Joyce et al.[38]	19	n.r.	Percutaneous, US	Radiotherapy	n.r.	8.1	8
Li, W. et al.[26]	50	22 (44)	EUS (44), Percutaneous, US (56)	Chemoradiotherapy	0 (0)	8.8	8
Li, Y. et al.[27]	137	n.r.	IO	Chemotherapy	0 (0)	9.4	9
Liu, K. et al.[28]	30	n.r.	Percutaneous, US	n.r.	n.r.	16	8
Lun et al.[51]	38	n.r.	Percutaneous, CT	Chemotherapy	n.r.	11.8	4†
Luo et al.[29]	35	n.r.	Percutaneous, CT	Chemotherapy	n.r.	9.5*	7
Mohiuddin et al.[30]	111	0 (0)	n.r.	Radiotherapy (30, 27%) [a] chemoradiotherapy (81, 73%) [b]	n.r.	7[a] 13[b]	3
Montemaggi et al.[39]	7	0 (0)	IO	Radiotherapy (4, 57%)	3 (43)	7	5
Morrow et al.[31]	33	9 (27)	IO	n.r.	8 (24)	8	4
Niu et al.[40]	60	n.r.	IO, CT	n.r.	0 (0)	10.4	6
Peretz et al.[41]	98	0 (0)	IO	Radiotherapy (27, 28%) [a] none (43, 44%) [b] chemotherapy (27, 28%) [c]	n.r.	6.2[ab] 10.3[c]	4
Schuricht et al.[32]	42	0 (0)	IO	Chemoradiotherapy	n.r.	12.8	8
Shipley et al.[42]	12	6 (50)	IO	Radiotherapy	3 (25)	11	6
Sun, S. et al.[43]	15	0 (0)	EUS	Radiotherapy (1, 7%)	4 (27)	10.6	8
Sun, X. et al.[33]	42	24 (57)	EUS	Chemotherapy	0 (0)	9	2

Reference	No. of patients	Metastasis *n* (%)	Application method	(Neo)-adjuvant therapy	≥Grade 3 complications *n* (%)	Median survival (months)	NOS ☆
Syed et al.[44]	18	1 (6)	IO	Radiotherapy	4 (22)	14	7
Wang, H. et al.[45]	28	0 (0)	IO, US	Radiotherapy (7, 25%) chemotherapy (10, 36%)	n.r.	10.1	4
Wang, J. et al.[46]	27	n.r.	IO, US	Radiotherapy (7, 26%) chemotherapy (6, 22%)	n.r.	8	4
Wang, W. et al.[47]	32	n.r.	Percutaneous, CT	Chemotherapy	0 (0)	14	6
Whittington et al.[34]	33	0 (0)	IO	Radiotherapy (13, 39%) [a] chemoradiotherapy (20, 61%) [b]	n.r.	5.5[a] 11.3[b]	8
Yang, M. et al.[48]	18	0 (0)	Percutaneous	Chemotherapy Radiotherapy (1, 6%)	3 (17)	7.3	5
Zheng et al.[49]	34	0 (0)	IO	Chemotherapy (8, 24%)	6 (18)	11	7
Zhongmin et al.[50]	31	12 (39)	Percutaneous, CT	Chemotherapy (10, 32%)	4 (13)	10.3	7
Total	1095	126 of 717 (17.6)	n.a.	n.a.	37 out of 612 (6.0)	n.a.	n.a.

*NOS, Newcastle-Ottawa Scale; IO, intra-operative; EUS, endoscopic ultrasound; US, ultrasound; CT: computed tomography; n.r., not reported; n.a., not applicable; †randomized controlled trial; *mean survival*

Phosphorus-32

P32 was first described to treat unrespectable pancreatic cancer in human patients as a directly injected brachytherapy in 1996.[52] In 2018 a new intratumoral therapy as microparticles (MP) was introduced by Harris et al. (2018) in the PanCO study.[53] Unlike I125, P32 decays by beta minus (β^-) emission with an energy of 1.709 MeV that penetrates tissue merely a few millimeters.[54] The half-life of 14.29 days offers a short term, higher dose rate when compared with I125 (59.4 days) for a given total dose. All included studies using P32 as intratumoral treatment for LAPC had a prospective design. Only one out of five studies compared P32 combined with neoadjuvant 5-Fluorouracil and adjuvant Gemcitabine chemotherapy versus chemotherapy alone in a RCT.[54] Three out of five studies injected colloidal chromic P32 (20-50 nm)[55] with or without macro-aggregated albumin (MAA) directly into the tumor.[52, 54, 56] MAA in combination with colloidal chromic P32 was used to overcome the high intratumoral pressure of high density pancreatic cancer to allow for injection and diffusion of the radioactive suspension.[52, 56] The colloidal chromic P32 was only injected percutaneously with CT guidance. The more recent MP brachy-therapy used an EUS application method instead.[19, 57] The results from this modality show large variations between complication rates and survival; and no clear causality was seen. With a median radiation dose of 1227 Gy after the initial dose delivery and up to six deliveries in 8 months, Rosemurgy et al. (2008) found the highest complication rate of 417% in 18 patients.[54] Because leakage of P32

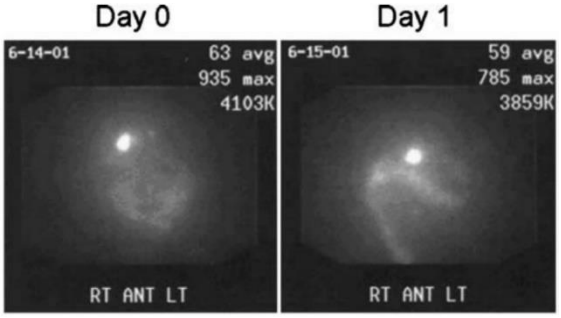

Figure 3 *Rosemurgy et al. (2008) showed high activity after injection of P32 into pancreatic cancer (Day 0) and high bowel activity the day after (Day 1). Hereafter, each injection was followed by a bowel cleanse.[45]*

to the duodenum resulted in a high bowel activity each injection was followed by a bowel cleanse (figure 3). Despite the bowel cleanse, complications rose with numeral direct causalities to a radiation overdose. Trial enrollment was abandoned after discouraging survival outcomes.[54] The list of complications confirms overdosing in this particular trial, with complications extending to the gastrointestinal (22 complications), pulmonary (4) and hematological (14) systems. The study by Westlin et al. (1997) also exceeded the more common 100 Gy ± 20% threshold with an accumulated median tumor dose of 11050 Gy (4400-19300 Gy).[19, 56, 57] Still, only one patient treated with 19300 Gy suffered tumor necrosis causing intestinal bleeding after multiple injections. Leakage to the gut was a common problem, as well as hepatic shunting. External leakage of the syringe could occur due to the high resistance in the tumor.[56] Regarding survival of the complete P32 treatment population, intratumoral treatment with 32P showed a survival between 5.2 and 16 months. Rosemurgy et al. (2008) showed the lowest survival with 5.2 months which was not prolonged when compared with chemo-therapy alone (11.5 months; p = .16).[54] The preliminary results from the recent study by Ross, et al. (2020) showed the highest survival with 16 months.[19] An overview of the study results of P32 brachytherapy are shown in table 2.

Table 2 *Results of Phosphorus-32 (P32) brachytherapy for locally advanced pancreatic cancer*

Reference	No. of patients	Metastasis *n* (%)	Intra-tumoral therapy	Application method	(Neo)adjuvant therapy	≥Grade 3 complications *n* (%)	Median survival (months)	NOS ☆
Bhutani et al.[57]	9	0 (0)	MP	EUS	Chemotherapy	27 (300)	n.r.	4
Order et al.[52]	47	19 (40)[a]	MAA + colloidal chromic P32	Percutaneous CT	Chemoradio-therapy	10 (21)	6.9[a] 12.0	8
Rosemurgy et al.[54]	18	0 (0)	Colloidal chromic P32	Percutaneous CT	Chemoradio-therapy	75 (417)	5.2	8†
Ross et al.[19]	42	0 (0)	MP	EUS	Chemotherapy	148 (352)	16	5
Westlin et al.[56]	17	n.r.	MAA + colloidal chromic P32	Percutaneous US	Chemotherapy (2, 11.8%)	3 (18)	7.6	6
Total	133	19 of 116 (16.4)	n.a.	n.a.	n.a.	263 of 133 (197.7)	n.a.	n.a.

NOS, Newcastle-Ottawa Scale; MP, microparticle; MAA, macroaggregated albumin; EUS, endoscopic ultrasound; CT, computed tomography; US, ultrasound; n.r., not reported; n.a., not applicable; †randomized trial

Immunotherapy

Immunotherapy is a relatively new form of cancer therapy and has firmly established itself as a novel branch in cancer care.[58] It is also used as a treatment for LAPC since the oldest study was published in 2001.[59] Cancer immunotherapy works by applying itself to certain cancer checkpoints and factors that may enhance natural cancer-fighting immune cells, prevent cancer development, enhance cancer cell detection or attack the tumor cell directly using oncolytic viruses. From the eleven immunotherapy studies included, seven injected modified viruses (see table 3).[60-66] These viruses selectively replicated in malignant cells and caused DNA damage inducing apoptosis. Three studies implanted enzyme producing cells which activated chemotherapy (P450)[59, 67] or induced tumor-antigen-specific CD8+ T-cells.[68] One study injected a double-stranded RNA oligonucleotide, called STNM01, that represses a specific tumor growth factor (CHST15).[69] They injected a volume of 16 ml in tumors from approximately 13.5 to 18 cm^3. The agent was injected at 16 locations in

tumor (1 ml each) with EUS, and a 22G needle.

Two studies compared chemotherapy alone versus chemotherapy with an oncolytic virus in RCTs.[63, 66] One used TNFerade adenovirus with 5-Fluorouracil chemotherapy and radiotherapy[66] and the other used H101 adenovirus with Gemcitabine[63]. The remaining studies had an open-label prospective design. Two studies used an angiographic approach in which encapsulated allogeneic cells were introduced into the tumor.[59, 67] The best approach to reach the tumor was through the inferior pancreaticoduodenal artery or the dorsal pancreatic artery.[59, 70] In each tumor, around 300 capsules of 0.8 mm diameter were placed. Out of 323 patients in eight immunotherapy studies, 247 (76.5%) severe (≥grade 3) complications occurred. The most frequent complications include leukocytopenia, severe pain, cholestasis, gastrointestinal bleeding, intra-abdominal infection, and deep vein thrombosis (DVT). The median overall survival ranged from 5.8 to 13.8 months. Again, the lowest overall survival was seen in a study that did not apply any systemic therapy.[69] Li et al (2011) found the highest overall survival with 13.8 months while more than half of the patients were diagnosed with distant metastasis.[62] The RCT by Herman et al. (2013) found a median survival of 10.0 months in both the virus and control group (p = .26).[66] The RCT by Xiao et al. (2011) found a median survival of 9 months in the virus group and 6 months in the control group (p = .004).[63] An overview of the study results of intratumoral immunotherapy are shown in table 3.

Table 3 *Results of intratumoral immunotherapy for locally advanced pancreatic cancer*

Reference	No. of patients	Metastasis *n* (%)	Immuno-therapy	Application method	(Neo)adjuvant therapy	≥Grade 3 complications *n* (%)	Median survival (months)	NOS ☆
Gong et al.[60]	9	n.r.	H101 adenovirus	EUS	Chemotherapy	n.r.	7	7
Hecht et al. (2003)[61]	21	12 (57)	ONYX-015 adenovirus	EUS	Chemotherapy	21 (100)	7.5	4
Hecht et al. (2012)[65]	50	0 (0)	TNFerade Biologic	EUS (54), Percutaneous (46)	Chemoradio-therapy	65 (130)	9.9	8
Herman et al.[66]	187	0 (0)	TNFerade Biologic	Percutaneous US/CT (92, 49%), EUS (95, 51%)	Chemoradio-therapy	116 (62)	10	8†
Hirooka et al.[68]	15	0 (0)	Dendritic cells	EUS	Chemo-immuno-therapy	4 (27)	11.5	8
Li, J.L. et al.[62]	15	8 (53)	p53 adenovirus	Percutaneous US	Chemoradio-therapy	8 (53)	13.8	7
Löhr et al.[59]	14	12 (86)	P450 cells	Angiography	Chemotherapy	14 (100)	10	5
Nishimura et al.[69]	6	5 (83)	STNM01 oligo-nucleotide	EUS	None	n.r.	5.8	5
Salmons et al.[67]	13	10 (77)	P450 cells	Angiography	Chemotherapy	16 (123)	9.5	3
Xiao et al.[63]	19	n.r.	H101 adenovirus	EUS	Chemotherapy	n.r.	9	6†
Yunwei et al.[64]	8	n.r.	H101 adenovirus	EUS	Chemotherapy	3 (38)	6	4
Total	357	39 of 306 (12.7)	n.a.	n.a.	n.a.	247 of 323 (76.5)	n.a.	n.a.

NOS, Newcastle-Ottawa Scale; EUS, endoscopic ultrasound; US, ultrasound; CT, computed tomography; n.r., not reported; n.a., not applicable, †randomized trial.

Combination therapy

Combination therapies consisted of at least two intratumoral therapies with or without (neo)adjuvant therapy. Only when combined with another intratumoral therapy, ablation was included. From the five studies included, two had a retrospective design that combined transarterial chemoembolization (TACE) with either I125 seed brachytherapy and/or RFA.[71, 72] TACE consisted of 100 mg/m^2 Gemcitabine and 100 mg/m^2 Oxaliplatin[71] or 1000 mg/m^2 Gemcitabine and 30-60 mg Cisplatin.[72] The remaining three studies consisted of prospective, open-label study designs.[40, 73, 74] These prospective studies all implemented I125 seed brachytherapy combined with either cryoablation or RFA. All intratumoral therapies were administered IO or percutaneous with either US or CT guidance. Described RFA methods consisted of a 17-gauge needle (1.15 mm) and aimed to ablate the complete tumor along with a 1.0 cm ablative zone or a diameter of 3 cm with a 2.0 cm ablative zone.[71, 74] When present (52.1%), metastasis were treated with either TACE[71], I125 or RFA[72], cryoablation[73] or chemotherapy.[74] Although the sample size is small, the two studies reporting severe complications showed relatively low severe complication rates with an average of 19.1%. The reported severe complications consisted of elevated liver enzymes, fatigue, pain, biliary leakage, and pancreatitis. The median overall survival ranged from 11 to 23 months. An overview of the study results of combination therapy are shown in table 4. A statistically significant difference (p = .026) was found between the survival of combination therapies with an average of 16.15 months (± 4.04 months standard deviation) when compared with the I125 brachytherapy, P32 brachytherapy and intratumoral immunotherapy with an average of 9.99 (± 2.71), 9.54 (± 4.40) and 9.09 (± 2.40) months, respectively. No significant difference in survival between the I125 brachytherapy, P32 brachytherapy and immunotherapy was found (p = .63).

Table 4 *Results of intratumoral combination therapy for locally advanced pancreatic cancer*

Reference	No. of patients	Metastasis *n* (%)	Therapy 1	Therapy 2	(Neo)adjuvant therapy	≥Grade 3 complications *n* (%)	Median survival (months)	NOS ☆
Das et al.[71]	47	64 (85)	TACE	I125 brachy-therapy	TACE for metastasis	9 (19)	23	8
	28			RFA		7 (25)	15.5	
Huang et al.[72]	71	51 (72)	TACE	I125 (34) RFA (22) RFA+I125 (44)	Chemotherapy for progressive metastasis	n.r.	11	8
Niu et al.[40]	32	0 (0)	I125 brachy-therapy	Cryoablation	n.r.	n.r.	13.7	6
Xu et al[73]	49	12 (24)	I125 brachy-therapy	Cryoablation	Chemotherapy (14, 29%) Chemo infusion (20, 41%)	n.r.	16.2	6
Zou et al.[74]	32	8 (25)	I125 brachy-therapy	RFA	Chemotherapy (26, 81%)	3 (9)	17.5	7
Total	259	135 of 259 (52.1)	n.a.	n.a.	n.a.	22 of 115 (19.1)	n.a.	n.a.

NOS, Newcastle-Ottawa Scale; TACE, transarterial chemoembolization; I125, Iodine-125; RFA, radiofrequency ablation; n.r., not reported; n.a., not applicable.

Intratumoral chemotherapy

Intratumoral chemotherapy was performed by four remaining studies including 71 patients. Three studies prospectively studied chemotherapy injection[75-77] and one studied a chemotherapy capsule implant vs systemic chemotherapy in a RCT.[78] Two studies used chemoradiotherapy[75, 76] and one used conventional chemotherapy[78] as adjuvant therapy. Only Li, J et al. (2016) did not report the complications.[78] This is unusual since they used a relatively large 12 F (4 mm) needle and used fibrin gel to prevent fistula and bleeding.[78] Yang, B. et al. (2017) used percutaneous CT-guided injection with a 8 cm 25-gauge (0.46 mm) needle in one location of the tumor.[77] This study first analyzed the intratumoral distribution of 1-2 ml of radiopaque agent before injecting chemotherapy with fibrin glue.[77] The three remaining studies reported that no severe complications occurred. Survival ranged between 9.0 months (n = 36) without metastasis[76] and 16.2 (n = 5) months with 80% metastasis.[77] The two remaining studies did not report metastasis. In the RCT no statistically significant difference (p = .07) was found in the survival between treatment with chemotherapy capsules (10.3 months) and systemic chemotherapy (8.1 months).[78]

Other intratumoral therapies

Palladium-103 (Pd103) seed brachytherapy was applied by two prospective studies in 1996.[79, 80] Pd103 is a lesser used gamma-emitter that has a similar half-life to P32 (16.99 days vs. 14.29 days) and a similar energy spectrum to I125 (20-35 KeV vs. 27-35 KeV).[79] Only one of the 26 included patients was diagnosed with distant metastasis. On average all patients were submitted to a dose of 110-124.2 Gy after IO implantation. All patients (n = 15) from Nori et al. (1996) received adjuvant chemoradiotherapy.[79] In the research group of Raben et al. (1996) five patients (45%) received chemoradiotherapy and two patients (18%) received adjuvant chemotherapy.[80] Raben et al. (1996) reported four (36%) severe complications including duodenal perforation, sepsis, cerebral vascular accident and radiation enteritis with a median survival of 6.9 months.[80] However, with a similar methodology to Raben et al. (1996), Nori et al. (1996) concluded a median survival of 10.0 months, however did not report if any complications occured.[79]

Nine patients without metastasis were treated with Iridium 192 (Ir192) intraluminal brachytherapy by EUS in a prospective study by Mutignani et al. (2002).[81] Ir192 decays by beta minus (ß-) emission with a similar halve-life with I125 (73.8 days vs 59.4 days). All patients received chemotherapy and three patients additionally received radiotherapy. Mutignani et al. (2002) reported one gastric ulceration and a median survival of 11 months.

Schad et al. (2014) retrospectively analyzed 39 patients who were treated with intratumoral Mistletoe (Viscum album L) therapy.[82] European Mistletoe induces antitumoral effects and immune stimulation with complementary chemotherapeutic side-effects. The Mistletoe was introduced to the tumor by EUS guidance with a 20-gauge (0.81 mm) needle. The agent was injected in fractions when retracting the needle from the distal tumor wall towards the tip of the scope. When retracting out of the tumor, Saline was injected to avoid backflow. In each session the tumor was injected at 1 to 3 times and the procedure was repeated every four weeks in combination with adjuvant chemotherapy. The median overall survival was 11 months without any severe complication.[82]

Intratumoral approach

Most therapies were performed through a percutaneous approach. In total, nine studies including 417 patients used a percutaneous approach to deliver an intratumoral treatment. An IO and EUS-guided approach followed closely with 11 studies including 399 patients and 12 studies including 208 patients, respectively. Two studies including 27 patients used an angiographic approach.[59, 67] The remaining 21 studies did not report intratumoral approach and/or complications. When comparing the chance of a severe complication per patient between these approaches, angiographically bears the highest risk with 111%, although the sample size is small (n = 27).[59, 67] EUS-guided and

percutaneous approaches contain similar risks for severe complications with 58% and 56%, respectively. While after IO administration, and thus an open approach, just 8% underwent severe complications. There was no clear causality between the complication rates and overall survival.

Discussion

The results of this systematic review contribute to the consideration that intratumoral therapies are safe and potentially effective to use in patients with LAPC.

Safety

Since most systemic therapies are quite invasive and pancreatic cancer has a high comorbidity, it was challenging to categorize complications related to the intratumoral therapy. Therefore, all severe complications were included for evaluation, also unrelated to the specific intratumoral therapy. Both grade 3 and grade 4 (Clavien-Dindo classification) complications were labeled as 'severe'. I125 brachytherapy being the most frequently studied and oldest therapy, 16 out of 27 studies reported complication rates and offered the lowest severe complication rate (6%; see table 1). Complication rates rose in the immunotherapy modality (76.5%), where 8 out of 11 studies reported the complication rates, and P32 brachytherapy (197.7%) in which all studies reported the complication rates. An explanation for the side effects and complications in immunotherapy is the assumption that an autoimmune toxicity may be triggered. The increased intra-abdominal infections and fever are a clear indication for this reaction and a common severe complication after immunotherapy.[83] Current research into upcoming immuno-therapies attempts to identify and control these side effects.[84] As mentioned in the results, patients who received P32 brachytherapy seemed to suffer from complications due to a radiation overdose. It may be discussed that due to the small penetration depth of β^- emission, overdosing has minimal effect on surrounding tissue if implanted correctly. This also explains how Rosemurgy et al. (2008), who suffered therapy diffusion into nearby tissues, found a high complication rate (417%) with a median radiation dose of 1227 Gy while Westlin et al. (1997) found a lower complication rate (18%) with a median tumor dose of 11050 Gy.[54, 56]
Other severe complications could (partially) be related to the invasive implantation of the intratumoral therapy or to systemic chemotherapy.
In almost all modalities that include systemic chemotherapy, severe side-effects occurred related to chemotherapy. Most common were leukocytopenia and thrombocytopenia which are known side-effect of chemotherapy for pancreatic cancer, especially for the now lesser used Gemcitabine.[85] The higher complication rate, complemented by a lower survival, was the main cause that gemcitabine was replaced with a combination chemotherapy of fluorouracil, leucovorin, irinotecan, and oxaliplatin (FOLFIRINOX).[86]

Survival

With regards to survival, the outcomes of immunotherapy (average of 9.1 months ± 2.4 standard deviation (SD)), I125 (10.0 ± 2.7 SD), P32 (9.5 ± 4.4 SD) and other intratumoral therapies (6.9-11.0) suggest roughly similar survival (p = .63 for immunotherapy, P32 and I125). It appears favorable to combine two intratumoral therapies (16.2 months ± 4.0 SD) instead of a single intratumoral therapy (p = .026 when compared with immunotherapy, P32 and I125). The given combinations may have had a cooperative and reinforcing effect on each other. Radiation from I125 brachytherapy increase permeability of irradiated tissue and improves the effect of chemotherapy.[87] In other combinations ablation was used to treat the mass of the tumor while brachytherapy was used to cover hard to reach locations and a broader margin.[40, 73, 74] However, these studies are more susceptible to bias, because only relatively strong patient can undergo multiple intratumoral therapies.
Metastasis may also be actively treated with local chemotherapy like TACE. Results show that when actively treating metastasis, systemic chemotherapy may even be omitted with favorable results.[29, 71, 73, 77] This is a notable advantage for patients with comorbidity who cannot receive surgical resection or systemic chemotherapy. In

China, TACE is already a standardized treatment method for patients with advanced pancreatic cancer.[88] Still, the limited vasculature and perfusion in pancreatic cancer may limit the therapeutic effect of TACE mono-therapy. The results show the importance to control metastasis closely, even when absent at diagnosis, because recurrent disease seems inevitable in all cases. Due to the insidious onset and probable microscopic spread at the time of diagnosis, pancreatic cancer is essentially a systemic disease.[32] Even if no metastasis was found, the disease may already have spread to the pancreatic surrounding. The results substantiate this theory by showing similar survival between studies with and without metastatic disease. More so, survival seems to be greatly influenced by the fact if systemic chemotherapy was administered or not.

Rombouts et al. (2014) already published a similar review to this one concerning ablative methods for LAPC. In the most common therapy group, RFA, he found an overall survival between 5.0-25.6 months.[14] The median survival of 25.6 months was found when RFA was combined with several different therapies including intra-arterial plus systemic chemotherapy. When RFA was applied as monotherapy, the median survival dropped to 14.7 months. Still, an evident selection bias was present.[89] More recent studies applying RFA for LAPC patients found a survival between 5.0-9.0 months with and without combination of chemotherapy.[90, 91] Overall, similar survival results can be found between RFA and most intratumoral therapies in this review.

Method application

When analyzing method applications, remarkable differences were found. I125 brachytherapy used seeds that have an adequate control of dose distribution because of their size (4-4.6 mm) and penetration depth (17 mm).[23, 24] The control of dose distribution in combination with a more researched therapy also results in a further advanced treatment planning when compared to novel modalities.[33] With therapies like P32 and Ir192, the penetration depth of beta- radiation is mere millimeters. Although healthy tissue close to the tumor is salvaged, it also makes complete tumor coverage more challenging and increases the risk of recurrence. In most other cases, regarding P32 brachytherapy, immuno-therapy, and intratumoral chemotherapy, the state of application was a suspension or fluid. The theoretical advantage of fluid-like application is the self-expanding range of the fluids within the tumor. However, due to the high-density characteristics of pancreatic cancer creating a high intratumoral pressure, this range is strongly limited. Two studies resolved this issue by injecting MAA into the tumor prior to the therapeutics.[52, 56] However, the precise mechanics of MAA to overcome intratumoral pressures in pancreatic cancer is still unclear. Another method to resolve the high tumor pressure is to make small suspension deposits in high quantities. Although this may be a time-consuming approach, it does offer a tumor specific treatment. If the therapy is visible on imaging, the therapy may be adjusted and optimized intra-operatively.

Methodological approach

Concerning the methodological approach, over the past decade a trend is visible towards less-invasive approaches like EUS-guided and angiographically placed therapies. Still, complication rates are high when comparing angiographically (111%), percutaneous (56%), or EUS-guided (58%) to an open approach (8%). However strongly influenced by the modality and additional (neo)adjuvant therapies, it can be argued that the higher severe complication rate in these seemingly less-invasive approaches are a consequence of the increased complexity. This creates a smaller margin for error of the less-invasive approaches. When a less invasive therapy contributes to a higher severe complication rate it may be presumed as counter effective. However, publications on these administration techniques were too limited and studies too heterogeneous to substantiate this claim. Safety and complication rates must be further analyzed in more frequently used repeated methodologies instead of small sample size feasibility studies.

Study limitations

Although study results seem promising, some limitations need mentioning. First, the definition of LAPC may vary between studies. As previously mentioned, LAPC in this study is often directly compared with LAPC according to the American Joint Committee on Cancer (AJCC) staging system.[15] However, the AJCC staging system does not include vascular involvement which is a main argument to define a tumor as unresectable. Also, comorbidity and patient preference may limit tumor resection. Therefore, some patients receiving intratumoral therapy in one study, would receive resection in another and thus improve clinical outcomes. Second, metastasis was present at different rates, locations, and quantities. Since no golden standard is available for LAPC it is difficult to extract a homogeneous control group from the population to define the intratumoral effects on survival. The missing golden standard also increases heterogeneity between the included studies, which makes a direct comparison challenging. Therefore, potentially successful intratumoral therapies or combinations of intratumoral therapies should be studied in large RCT's, until a golden standard becomes available.
Although an overview is given, results were strongly limited in this review. Sample sizes were often small and non-randomized and selection bias was prominent. It is therefore important to take the NOS into account when reviewing the study results. The broad variety of included studies may have limited method specifications on account of clarity of this review. The broad variety of all included modalities limits the evaluation of technical specifications. Furthermore, (neo)adjuvant therapies have been categorized under type of therapy (e.g. chemotherapy, chemoradiotherapy, radiotherapy, etc.) and not on technical aspects, start, duration and iteration of the therapies. The (neo)adjuvant therapies also varied widely and therefore could not be compared. Many studies did not include pain scores, quality of life or WHO scores as outcome. These results were therefore excluded from this review. This amplifies uncertainty if the increase in survival actually does benefit the patients and those close to them.

Conclusion

Finally, a wide variety of intratumoral therapies is described and an overview is reported. Although evidence is limited to case series and cohort studies most intratumoral therapies seem feasible and safe as treatment for patients with locally advanced pancreatic cancer. Combined intratumoral therapy may have the best survival benefit for patients who are ineligible for resection when compared with single intratumoral therapy. Notably, randomized trials need to be performed to substantiate the advantage of intratumoral therapies.

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*Appendix A-C are unavailable in this thesis version. Please contact the author for further information

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